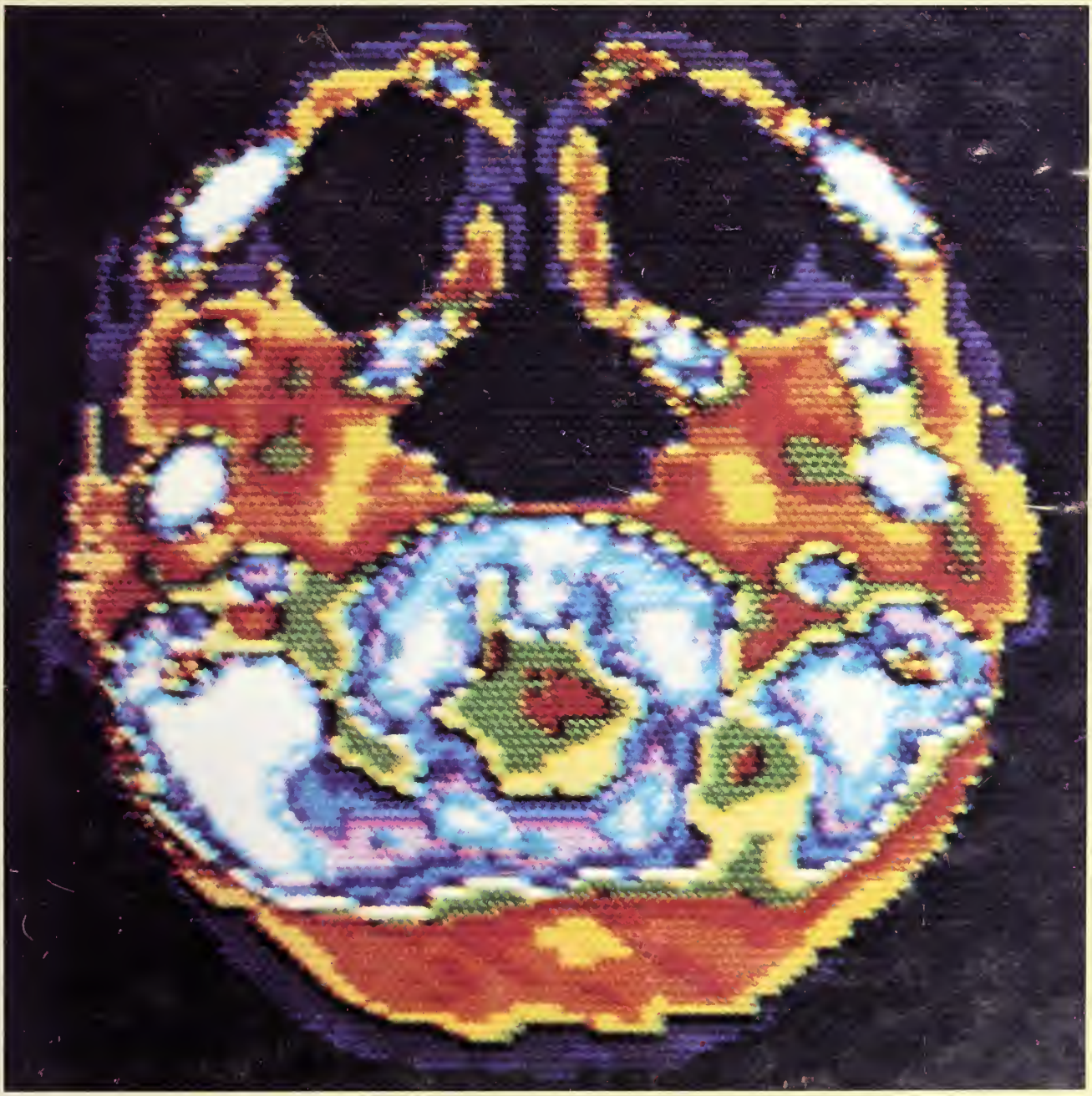
Research ADVANCES

1975



Cover Photograph

Computerized axial tomography image of the skull base of a live human patient forms a striking pattern. The modified X-ray scanner (ACTA-Scanner) developed at Georgetown University Medical Center, with support of the National Institute of Neurological Diseases and Stroke, provides diagnostic information for evaluation of the cervical spinal cord or other soft tissues.

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The Critical Mass Concept in Biomedical Research

The principal contribution of the National Institutes of Health, as the agency of the Federal Government assigned lead responsibility for biomedical research, has been expansion of the body of knowledge incorporated in medical science. The national progress against disease achieved since World War II is a consequence of this comprehensive broadening of the general research base. It is important to note that this advancement of knowledge is a whole that is greater than the sum of its undeniably important parts, the individual achievements of American scientists.

It would be impossible at any time to list all contributions of the staff scientists and the non-Federal investigators supported by NIH grants and contracts. A recounting of specific advances attributable to the creativity of these men and women is, however, appropriate every year. This volume attempts to fulfill that purpose for Fiscal Year 1974 and the final months of Fiscal Year 1973. It is offered with the hope that understanding of NIH contributions to biomedical knowledge will be enhanced.

Federal nurture of health-related science has revolutionized the range of diagnostic, therapeutic and preventive capabilities available to the American people.

Over the years it has transformed the prognostic expectancy in heart conditions, some cancers, cerebrovascular disorders, the infectious diseases, mental illness, and many other afflictions.

It has opened penetrating insights into the nature of life, the functioning of biological systems, the basic character of disease, and the degenerative and developmental processes.

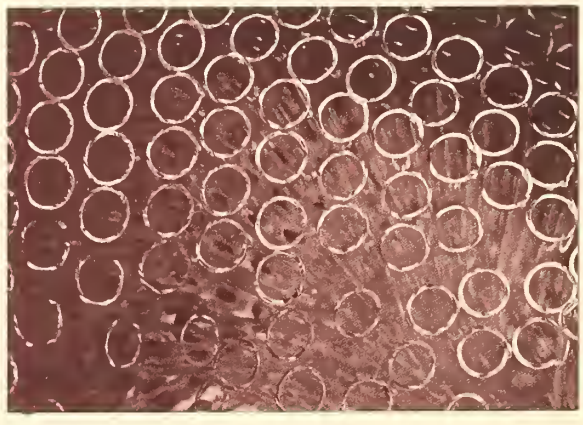
American society allots about 4 percent of the amount it spends for all health purposes to medical research and development. The Federal Government's share of these research expenditures was about two-thirds of the national total in Fiscal Year 1974, and of this portion the National Institutes of Health budget of $1.8 billion was about 64 percent.

The programs of the National Institutes of Health have undergone dramatic growth over the past quarter century, both in the size of Congressional appropriations and in the accomplishments attributable to application of these funds. NIH intramural research has achieved world renown in continuing productivity, high standards of achievement, and a rarely matched *esprit de corps* within the scientific staff. The NIH approach, exploiting interdisciplinary and intercategorical collaboration, has demonstrated the power resulting from the assembly of a critical mass of talent.

Administration of the intramural program promoted many pioneering procedures now widely accepted, including selective breeding of animal models for individual disease studies, application of radioisotopic techniques to the charting of metabolic and biochemical pathways, development of tissue culture technology, application of computerized manipulation of data, the extension of research to ambulatory out-patient facilities, the application of ethical standards to research involving human subjects, and the optimization of research training.

The NIH leadership in fundamental studies has been demonstrated over the years in the explosion of understanding in molecular biology, discovery and evaluation of the anti-hypertensive effects of the biogenic amines, the identification of respiratory viruses, testing of synthetic cortico-steroids, clarification of the theory of infections and of the nature of dental caries.

Notable categorical advances resulting from basic research include dramatic progress in the chemotherapy of cancer, new treatments for hypertension, improved surgery of the heart, elucidation of the handling of insulin by diabetics, isolation of multiple viral causes of the common cold, and the development of a vaccine against rubella.



Others, selected at random, are illustrative of the expansion of the knowledge base upon which applications have already begun to appear, or may be expected in the future, including:

Discovery by a multidisciplinary team that a definite genetic component underlies schizophrenia and related illness.

Essential discoveries leading to subsequent exposition of the causes of phenylketonuria, a hereditary form of mental deficiency.

Tracing of the ion exchange mechanisms and explanation of the transfer of chemicals within the kidney. This knowledge led to the current theory of urinary dilution and concentration, essential to present understanding of the function of the kidney in health and its malfunction in disease.

Measurement of physiological and pathological forces on the left side of the heart, making possible vast improvement in the diagnosis of cardiac disease.

Classification of the hereditary abnormalities of blood lipids, which provides the basis for current investigation of atherosclerosis.

Disclosure of a substance in the blood, first called the Australia antigen, which thereafter was proved to be the causative agent and principal diagnostic indicator of serum hepatitis.

Identification of the enzymatic bases for a remarkable number of genetically determined metabolic diseases, which led to effective treatments, diagnostic tests, and prevention of some of these disorders.

Discovery of the adenoviruses, an important family of viruses causing numerous respiratory illnesses, and of a variety of other "new" viruses, particularly those related to the induction of cancer.

Perfection of a new and promising technique leading to possible live virus influenza vaccines by developing a temperature-sensitive (defective) virus strain and mating it with the Hong Kong influenza virus.

Basic studies establishing the scientific base for community fluoridation of water supplies for the prevention of tooth decay.

Fundamental research contributing to the understanding of the causes of dental caries, providing hope that this disease can be eliminated.

Development of reliable techniques for immunological detection and the quantitation of tumor viruses, and of several new drugs useful in the treatment of human cancer, providing for the recent achievement of probable cures of several forms of cancer.

Intramural research is the smaller part of the NIH effort, however. Federal funding has favored NIH support of extramural research, in most years by a ratio of four to one or greater. Formerly, grants-in-aid were usual, but recently increasing use has been made of the contract mechanism for targeted and applied efforts. In contrast to intramural operations, which have grown with relative modesty, support of extramural research over the years has expanded rapidly in response to public and Congressional interest. Nearly 175,000 non-Federal scientists have been supported by this NIH outreach to more than 10,000 different institutions.

Qualitatively, as well, the record is a proud one, and it is both factual and appropriate to note that the overwhelming majority of all that is good in American biomedical research has been supported by the National Institutes of Health. Concentration on excellence and an unsurpassed level of productivity have been integral parts of research supported by NIH in every important center of the nation and in many other countries. The scope and constancy of NIH support unquestionably broadened the base of all research and created conditions in which it flourished.

Here, too, it can be said that a critical mass has been assembled and perpetuated in numerous distinguished centers. And in a very real sense NIH-funded biomedical science constitutes a broader critical mass sustaining and contributing to all American scientific accomplishments.

This inventory of advances is intended to demonstrate the impact of the assembly of research talent, in Bethesda and nationally, on the knowledge base prerequisite for progress toward better national health.

Thermography may indicate existence of breast disease by difference in heat pattern of two breasts.

Synthesis of Artificial Gene

A living cell is a delicately controlled system of proteins, carbohydrates, nucleic acids and smaller molecules, all normally acting in harmony as the cell proceeds through cycles of growth, mature function, and division. In a cancer cell, however, the controls seem to break down. The cell never matures and instead proceeds directly from growth to division, producing a spreading mass of similar cells that displaces normal cells and—if left unhindered—eventually threatens the host's life.

A growing body of research indicates that aberrations in cellular controls lie at the heart of the cancerous process. Cancer cells may produce biochemicals normally produced by healthy, immature cells of the embryo or fetus. Other by-products of cancer cells may be molecules such as hormones, normally secreted by the mature and highly differentiated cells of glands. Experiments with chemicals that affect control mechanisms have yielded tantalizing clues that normal controls may be restored, reversing the cancerous transformation.

In one approach to understanding the mechanisms that control cell growth and multiplication, Dr. H. Gobkind Khorana and coworkers at the Massachusetts Institute of Technology, Cambridge, are working toward the synthesis of a functioning gene, complete with the control elements that start and stop the gene's function. Nobel Laureate Khorana has been a grantee of the National Cancer Institute (NCI) since 1958.

The genes in a cell nucleus direct the synthesis of proteins and other molecules that determine the cell's characteristics. Each gene is a chain of deoxyribonucleic acid (DNA) units, the molecular building blocks used by the MIT scientists to construct an artificial gene.

Dr. Khorana announced achievement of an important goal at a meeting of the American Chemical Society in August 1973. He reported that he and his colleagues had synthesized a potentially functioning gene of 126 DNA units, using laboratory methods developed over many years. The scientists duplicated the arrangement of DNA units in a gene that directs the formation of a particular ribonucleic acid (RNA) molecule in a bacterium called *Escherichia coli*. The RNA, called tyrosine transfer RNA, is responsible for incorporating an amino acid, tyrosine, into proteins as they are synthesized in the living cell.

The MIT scientists are currently working on the synthesis of additional DNA chains that will be atached to each end of the gene to start and stop the formation of RNA. These two control groups each may be 20 to 30 DNA units long. (In August, 1974, they reported they had completed the "stop" signal and were in the process of attaching it to the gene.)

Dr. H. Gobind Khorana, NCI grantee at the Massachusetts Institute of Technology, synthesized artificial gene.

When the entire gene is complete, Dr. Khorana intends to demonstrate that the gene is functional by inserting it in a mutant bacterium that lacks the ability to synthesize tyrosine transfer RNA correctly. A virus that infects bacteria can be used to carry the artificial gene into the bacterium's genetic apparatus. If the artificial gene is functional, the scientists expect to see the appearance of normal proteins in the bacteria.

The complete gene can be used to study the mechanisms that control its function in synthesizing RNA. Studies by scientists at NCI and other laboratories have indicated that the initiation and termination of RNA synthesis depends on interactions of the DNA control groups with RNA polymerase, the protein that stimulates synthesis of RNA chains from individual RNA molecules. Other small molecules may be necessary as cofactors in regulating the RNA synthesis.

The artificial gene may provide a well-defined system for studies of gene function and control. By deliberately changing the composition of DNA units in the control elements, for example, scientists may learn more about how these gene segments interact with proteins and other factors that determine whether the

gene is turned "on" or "off." From such studies may come a new understanding of how genes are controlled—and perhaps of how genes can get out of control.

Cyclic AMP

Cyclic adenosine monophosphate (cyclic AMP) is one of the biochemicals known to play a role in cellular control mechanisms. During 1973, one group of NCI scientists studied changes in cyclic AMP levels within cells made cancerous by viruses. Other NCI scientists found that a molecule similar to cyclic AMP can cause the disappearance of mammary tumors in rats.

Cyclic AMP is synthesized within the membranes of normal cells when hormones interact with the membranes' outer surfaces. Cyclic AMP molecules may then act by a number of possible mechanisms to control the function of genes or gene products. Cyclic AMP thus acts as a critical link in the hormonal mechanism that maintains a balance in the functions of many different body cells.

Dr. Ira Pastan and colleagues have shown that the amount of cyclic AMP in laboratory-grown cells will decrease when the cells are made cancerous by viruses. The cells regain some—but not all—of the characteristics of normal cells when treated with one of several compounds similar to cyclic AMP or with chemicals that increase levels of cyclic AMP within the cells.

In the *Proceedings of the National Academy of Sciences, USA,* the NCI group reported that the cancerous transformation of chicken embryo cells by a particular strain of virus is accompanied by a decrease in the activity of adenylate cyclase, the membrane-bound enzyme that synthesizes cyclic AMP from adenosine triphosphate (ATP). The scientists used the Bryan high-titer strain of the Rous sarcoma virus, an RNA-containing virus that causes cancer in chickens.

A temperature-sensitive mutant of this virus, active at 37 degrees Centigrade but inactive at 41 degrees, strengthened the evidence relating viral transformation, cyclic AMP levels, and enzyme activity. When normal-appearing, virus-infected cells at 41 degrees were cooled to 37 degrees, the cells simultaneously changed to a cancerous appearance, while cyclic AMP levels dropped and the activity of adenylate cyclase decreased.

The scientists went on to study the properties of adenylate cyclase in virus-transformed cells. They found that the enzyme became less able to bind ATP and magnesium, which are necessary factors in the synthesis of cyclic AMP.

In an additional paper, in *Biochemical and Biophysical Research Communications,* the team reported that a second strain of Rous sarcoma virus produced a decrease in the activity of adenylate cyclase by a different mechanism. Their evidence indicated that the enzyme's binding of ATP and magnesium remained unaffected when the enzyme's activity decreased.

The investigators suggested that the second virus—the Schmidt-Ruppin strain—may decrease cellular levels of an unidentified activator of the enzyme. Because the enzyme is an intimate part of the cell membrane, however, the scientists also noted that both of the viruses might cause changes in the membrane that would in turn be responsible for the decrease in adenylate cyclase activity.

Another group of NCI scientists, headed by Dr. Pietro M. Gullino, reported at a conference on mammary (breast) cancer that dibutyryl cyclic AMP blocked the growth of several different cancers in rats. Dibutyryl cyclic AMP is one of the cyclic AMP derivatives found by Dr. Pastan and others to raise cyclic AMP levels within laboratory-grown cells.

Dr. Gullino and his coworkers found that dibutyryl cyclic AMP halted the growth in rats of several types of mammary tumors that depend on hormones for growth, as well as a hormone-independent mammary tumor and a liver tumor. The effect was not seen with all tumors tested, however. The compound only slowed the growth of a mammary tumor induced by a chemical called nitrosomethylurea, and had no effect on the growth of a tumor of connective tissue.

Continued treatment with dibutyryl cyclic AMP produced a complete disappearance of the hormone-independent mammary tumor—the Walker 256 carcinoma—in some of the rats. Upon further study, the scientists found that the Walker 256 carcinoma has at least two types of cells, one responsive to dibutyryl cyclic AMP and the other unresponsive. Rats whose tumors disappeared apparently had cells of the responsive type.

Other observations indicated that cyclic AMP levels in the cells might not be directly related to the action of dibutryl cyclic AMP in living

animals. The dibutyryl compound increased cyclic AMP levels in both responsive and unresponsive Walker 256 cells, whereas only the former cells stopped growing and eventually disappeared. The scientists are looking for other differences between the two types that might explain why dibutyryl cyclic AMP had an effect on only one of them.

Diet and Colon Cancer

The relationship of diet to the increased occurrence of colon cancer among Hawaiian residents of Japanese descent has been under intensive study for several years. Investigators led by William Haenszel of NCI and Dr. Mitsuo Segi of Tohoku University School of Medicine, Japan, reported that the relatively large amount of beef in the Westernized diet of these Hawaiian colon cancer patients may be linked to their development of the disease.

Past studies of emigrants to the U.S. or Australia from Western Europe—where the incidence of colon cancer is intermediate between rates in Japan and the U.S.—indicated that the shift to higher rates occurred within two generations after migration. This finding suggested that agents in the new environment contributed to the increase.

As early as 1967, Dr. Enrest L. Wynder of the American Health Foundation, New York City, and his colleagues reported that colon cancer patients in Japan consumed a more Westernized diet than comparable persons without this type of cancer.

In the most recent research, NCI-supported scientists studied 179 colon cancer patients and 357 patients with noncancerous diseases. These patients were either Japanese immigrants of 20 years residency or more in Hawaii, or Hawaiian-born persons of Japanese ancestry.

The scientists interviewed all 536 persons to study the patterns of their migration to Hawaii, their dietary habits, living customs, occupations, and medical and reproductive histories. They found, in agreement with previous studies, that the colon cancer patients had higher rates of intestinal problems such as constipation, hemorrhoids and polyps.

A significant number of the colon cancer patients interviewed revealed that they no longer ate Japanese-style meals. Instead, they had adopted a diet consisting largely of beef, legumes such as beans, and refined carbohydrates such as starches. The rate of colon cancer for persons who no longer ate Japanese-style meals was about twice that of persons whose diet had remained relatively unchanged.

The scientists found that the greater quantity of beef was the most apparent difference between the diets of colon cancer patients and noncancer patients. Far from claiming that beef consumption causes colon cancer, however the team suggested that further studies are necessary to identify the causative factors in this disease, at least one of which may be closely associated with beef.

Screening for Cancer-Causing Agents

In the National Cancer Institute's intensive effort to identify cancer-causing chemicals in our environment, approximately 445 suspect chemicals are being investigated. These include industrial chemicals, pesticides, food additives and drugs, as well as natural substances. Tests are conducted at 20 laboratories throughout the U.S., including the Frederick Cancer Research Center at Fort Detrick, Maryland.

Dr. Joseph A. DiPaolo uses cell culture system to identify possible cancer-causing agents

At present, evaluation of each chemical requires two to three years and more than 500 laboratory animals at a cost of approximately $75,000. Several different animals are being exposed to chemicals and monitored for tumor development.

For such extensive testing, NCI must consider many variables before selecting a chemical for study. Scientists carefully weigh the extent of human exposure to the chemical, the amounts in which it is produced and used, and preliminary data on its toxicity. Because humans are exposed to tens of thousands of environmental chemicals each year, NCI is supporting research to develop faster and less expensive test systems.

NCI-supported scientists continued to develop systems of laboratory-grown cells that undergo cancerous changes when exposed to cancer-causing chemicals. Dr. Joseph DiPaolo and his colleagues at NCI reported progress on their cell culture system in the *Archives of Pathology*. The new method, which may indicate cancer-causing activity in as little as two weeks, is a modification of an earlier technique developed by Dr. DiPaolo and scientists at other institutions.

The NCI scientists first injected a test chemical into pregnant hamsters. Later, they removed the embryos from the hamsters and grew cells of the embryo in test tube cultures. By injecting the chemical into a living animal the scientists exposed the embryo cells both to the chemical and to its metabolic by-products.

The scientists detected cancerous transformation by viewing the cells under the microscope. If cancer-like, the cells were grown in quantity and injected into healthy hamsters. Appearance of tumors in the animals confirmed that the cells were indeed cancerous.

The scientists tested 12 chemicals known to cause cancer, as well as five that do not. For each test, they used four pregnant hamsters with average litters of 12 to 14 embryos. Each test correctly identified the cancer-causing potential of the test chemical.

Much further work is planned by NCI using samples of unidentified chemicals in blind trials to determine if the method consistently detects cancer-causing activity.

The cell culture systems being developed may aid the Institute in selecting suspect chemicals for further study. Eventually, a series of such tests may be used both to screen rapidly for suspect chemicals and to confirm their cancerous potential.

Many factors must be considered in evaluating the emerging tests, however. Some chemicals, for example, require enzyme activation before they become carcinogenic. Others do not cause cancerous changes themselves but metabolize in the animal to other chemicals believed to be primary agents of tumor formation. Unidentified differences between humans and test animals also may determine whether a chemical fulfills its cancerous potential.

Results from any of the tests, either those in live animals or in cultured cells, can predict cancerous potential for humans only by analogy. Consequently, NCI is developing other test systems using cultured human cells in an effort to predict more directly the effects of environmental chemicals on man.

Viruses and Human Cancer

For many years, scientists have carefully documented evidence that viruses can cause cancers in animals. The evidence for virus causation in human cancers remains circumstantial, but new clues are emerging.

Broadly speaking, virsuses are of two types: those with chemical cores of DNA (deoxyribonucleic acid) and those with RNA (ribonucleic acid) cores. These chemicals comprise the hereditary information carried by the virus. Both RNA and DNA viruses cause animal cancers. Both appear to have at least some role in one or more human cancers.

Reports by NCI-supported scientists during 1973 continued to demonstrate the presence of incomplete RNA virus information in some human breast cancers and leukemia. At an international symposium in Japan, a team of scientists led by Dr. Sol Spiegelman of Columbia University College of Physicians and Surgeons, New York City, and Dr. Jeffrey Schlom of NCI reported on biochemical studies. They found similarities between purified cores of RNA from the mouse breast cancer virus and virus-like particles found in some samples of human milk. The scientists also found that human breast cancer cells contained hereditary information resembling that from the mouse virus.

Biochemical studies of this type indicate only that viral genetic information related to that in animal cancer viruses can be found in some human cancer cells. How the information got into the cells and whether it does anything to

or for the cells remain to be determined.

Additional evidence for the presence of cancer virus components in human leukemia cells was reported by scientists from NCI and Litton Bionetics, Inc., Bethesda, Maryland. The scientists, led by Drs. Robert C. Gallo of NCI and David H. Gillespie of Litton Bionetics, reported that leukemic white blood cells from some adults contained genetic chemicals closely related to viruses that cause cancers in mice and monkeys. Again, specific questions about the role of the virus in the origin or maintenance of leukemia in adults remain unanswered.

The scientists conducted extensive tests of the hereditary information found in the leukemic cells of several adults with acute forms of the disease. The leukemic cells were separated from other portions of the blood, the cellular membranes removed, and the internal components of the cells were separated. The portions having a density similar to that of known cancer-causing RNA animal viruses were purified repeatedly to extract an enzyme associated with cancer virsuses.

This enzyme, called reverse transcriptase, previously was reported by scientists from the same laboratories to be biochemically identical to that of known animal RNA cancer viruses. Drs. Gallo and Georga Todaro of NCI showed the enzyme to be closely related immunologically to enzymes of two non-human primate viruses, those of the woolly monkey and gibbon ape. Dr. Gallo also has shown that antibodies to normal human cell enzymes do not inhibit the viral enzymes, including the one in the human leukemic cells.

Using the RNA from the human leukemic cells, and the human reverse transcriptase, the NCI-Litton team constructed a DNA copy of the RNA. They then determined that about half of the DNA product was related to RNA isolated from mouse and monkey cancer viruses. This is strong evidence that the RNA isolated from the human leukemic cells is related to RNA cancer viruses.

In discussing their findings, the scientists suggested that the postulated human cancer virus may be an incomplete entity within the human cells rather than a complete virus. Further, they suggested that the viral-like entity may be similar to certain animal solid tumor viruses which exist in a hidden state in animal cells.

DNA Viruses and Human Cancer

A human DNA virus, the Epstein-Barr virus, has been implicated in certain human cancers. This virus, which exists naturally in large segments of the human population, has been studied as a possible cause of Burkitt's lymphoma, a cancer of the lymph system chiefly affecting African children, and nasopharyngeal cancer, a cancer of the nasal passages. More recently, the virus has been shown to cause infectious mononucleosis, an acute non-malignant infection of the lymph glands that often affects young adults. There is no known association between infectious mononucleosis and cancer.

The link between the Epstein-Barr virus and the cancers originally was based on the high levels of antibody to the virus observed in patients with either Burkitt's lymphoma or nasopharyngeal cancer. Antibodies are produced by a person's immune system in response to invasion by bacteria, viruses, or other foreign substances. Scientists also found that the appearance of higher levels of antibody to other proteins of the virus correlated with a good prognosis for Burkitt's lymphoma patients who had been treated with anticancer drugs. Biochemical analysis of cancer cells from patients with either type of cancer showed that the cells contained segments of the virus core.

Recently, NCI-supported scientists developed a new technique that provided additional evidence linking the virus with these two cancers. Drs. Beverley M. Reedman, Queensland Institute of Medical Research, Australia, and George Klein, Karolinska Institutet, Stockholm, reported in the *International Journal of Cancer* that, using an immunologic technique, they could attach a fluorescent label to a protein product of the Epstein-Barr virus in the nuclei of nearly all cancer cells tested from Burkitt's lymphoma patients. The virus-specific protein was associated with the chromosomes in the nuclei, or hereditary centers of the cells. Dr. Hans Wolf and associates at the Universitat Erlangen-Nurnberg, Germany, later reported in *Nature* that they had used the technique to detect the virus in nasopharyngeal cancer cells.

The test now is used as a marker for the presence of the hereditary core of the Epstein-Barr virus in the nuclei of cells. So far, the marker has been found only in Burkitt's and

nasopharyngeal cancer cells. The marker was not found in cells from breast cancer, myeloma, brain tumor, cells from an inflammation of the lining of the lungs or normal cells from the nervous system. The scientists also have failed to find the marker in cells from marmosets carrying a similar monkey DNA virus that causes lymphoma in marmosets and owl monkeys.

In research partly supported by NCI, scientists from the Yale University School of Medicine reported that in monkeys the Epstein-Barr virus can cause a disease resembling human malignant lymphoma. Drs. Thomas Shope and colleagues reported in the *Proceedings of the National Academy of Sciences* that one of four marmosets inoculated with cells made cancerous by the virus and three of four marmosets inoculated directly with the virus developed lymphoma within 31 to 46 days after inoculation. In a concurrent issue of the *International Journal of Cancer,* other NCI-supported scientists reported that an owl monkey inoculated with the virus also developed malignant lymphoma.

These studies do not prove that the Epstein-Barr virus causes cancer in humans. Instead, they raise additional questions about the virus, such as why it should be linked so closely only to African Burkitt's lymphoma and not to very similar lymphomas in other parts of the world. A related question is why the same virus should be linked just as closely to nasopharyngeal cancer, a disease that affects all age groups in many parts of the world.

The scientists are using many approaches to answer these questions. For example, they are studying whether a specific type of cell is susceptible to the virus, whether a specific variant of the virus is responsible for their findings in human cancer cells, whether heredity plays a role, whether another agent may work with the virus to cause cancer, and whether, indeed, infection by the virus is necessary for the cells to become cancerous.

Immunologic Reactions in Cancer Patients

A team of scientists from NCI, Litton Bionetics, and the George Washington University Medical Center has found evidence for an antigen common to patients with lymphomas, nasopharyngeal carcinoma and acute leukemia. The evidence came from positive skin reactions when the patients were tested with an extract from cancer cell membranes.

Other NCI scientists have found that a possibly different antigen may be shared by cells from patients with Burkitt's lymphoma and acute leukemia. Results from both studies may lead to advances in cancer detection and treatment.

The research using skin reactions was conducted by Drs. Ronald B. Herberman and associated scientists of NCI, in association with Dr. James L. McCoy of Litton Bionetics, Inc., Bethesda, Maryland, and Dr. Ariel C. Hollinshead of the George Washington University Medical Center, Washington, D.C. Dr. Heberman reported results of their studies at the Sixth International Symposium on Comparative Leukemia Research, held at Nagoya/Ise-Shima, Japan.

Patients with acute leukemia, lymphomas and a number of other cancers were tested for immune reactivity with crude membrane extracts from several types of human lymph cells grown in the laboratory. These cells came originally from patients with acute leukemia or Burkitt's lymphoma, and from normal adults. The cells from the Burkitt's lymphoma patient have been named RAJI cells. When the investigators applied an extract of the laboratory-grown cells to a patient's skin, a positive skin reaction, such as thickening of the skin or a small lump, indicated that the patient previously had encountered an antigen included in the cell extract.

The extracts from laboratory-grown leukemia and RAJI cells elicited positive reactions in patients with acute leukemia and lymphomas, including Hodgkin's disease. Dr. Levine also found positive reactions in Chinese patients with nasopharyngeal carcinoma (cancer of the back of the nose and throat) studied in Hong Kong and Singapore.

Extracts from RAJI cells produced positive skin reactions in more than one-third of 65 acute leukemia patients while they were in remission, that is, free from all evidence of disease. The extracts also produced positive skin reactions in about one-half of 25 patients with lymphomas, including both Hodgkin's disease and other cancers of the lymph system. Skin reactions were negative in about 20 other cancer patients, including those with cancers of the breast and larynx.

Extracts of the cells from normal adults produced uniformly negative reactions in all cancer patients.

These results indicate that many patients with acute leukemia, Hodgkin's disease and other cancers of the lymph system, and nasopharyngeal cancers have developed an immune reaction against the same antigen. Furthermore, this antigen is associated with membrane extracts of laboratory-grown lymph cells from patients with acute leukemia and Burkitt's lymphoma.

As already discussed, a number of investigators have found antigens of the Epstein-Barr virus (EBV) in laboratory-grown cells from patients with Burkitt's lymphoma and nasopharyngeal cancer. Dr. Herberman and his colleagues detected antibodies to EBV antigen in blood samples from most of the lymphoma patients who gave positive skin reactions in his tests.

However, the common antigen in the NCI studies may be associated with an agent other than EBV. Other studies have not linked EBV, a deoxyribonucleic acid (DNA) virus, with leukemia. Most evidence points instead to ribonucleic acid (RNA) viruses as causes of leukemia in animals. In addition, the normal adult cells used in the NCI study had an EBV antigen, but failed to produce positive skin reactions.

Dr. Hollinshead has isolated the antigen from membrane extracts of cells of patients with acute myelocytic leukemia. In the future, Dr. Hollinshead and the NCI scientists plan to prepare antibodies to the antigen, which may provide a simpler way to detect the antigen than the present skin tests.

Tests for the antigen also may be useful in following the progress of treatments. Dr. Herberman observed that the skin tests were positive in more than one-third of the acute leukemia patients in remission, whereas the skin tests were negative in most of the patients in whom the disease had recurred.

In other research at NCI, Dr. Dean L. Mann and others have found that lymph cells (white blood cells) from patients with acute lymphyocytic leukemia or acute myelocytic leukemia have antigens which make the cells vulnerable to destruction by antibodies produced in rabbits injected with laboratory-grown RAJI cells, the same type of cells used in the studies by Dr. Herberman.

Acute lymphocytic leukemia is the form of leukemia most common in children; acute myelocytic leukemia occurs primarily in adults. At the time of Dr. Mann's report at the National Conference on Virology and Immunology in Human Cancer, New York City, the antigens had been detected on white blood cells of all 47 patients tested.

In the first part of their study, the scientists found that antibodies in the blood of the RAJI-cell treated rabbits caused a measurable destruction of human acute leukemia cells in a test-tube reaction. The scientists then injected RAJI cells into the skin of 6 acute leukemia patients and found that 4 (two-thirds) of the patients developed an antibody in their blood similar to the antibody produced in rabbits.

In subsequent laboratory experiments, blood specimens from these patients destroyed leukemia cells obtained prior to treatment, as well as RAJI cells and leukemic cells from other acute leukemia patients. Efforts are under way to determine whether patients treated with RAJI cells will be able to destroy their own leukemia cells consistently.

Dr. Mann reported that the rabbit antiserum reacted only with acute leukemia cells and RAJI cells in tests so far. The antiserum did not react with laboratory-grown cells from human cancers of the cervix, breast, lung or bone. The antiserum also failed to detect the antigen or antigens in tests with normal white blood cells, normal bone-marrow cells, immunologically-stimulated normal white blood cells, human embryo cells, or with white blood cells from patients with infectious mononucleosis, chronic lymphocytic leukemia or myelocytic leukemia.

The antiserum test also may be valuable in monitoring the course of treatment of acute leukemia patients. The scientists could no

longer detect the antigen when drug treatment caused a disappearance of leukemia symptoms. The antigen had been detectable initially in all patients tested. (In comparison, the skin tests of Dr. Herberman and his colleagues indicated a response in about one-third of acute leukemia patients made free of disease by treatment.)

Dr. Mann could detect the antigen again, however, on cells from patients who suffered a recurrence of the disease. The antigen generally appeared in advance of other symptoms, providing an earlier warning than previously available that treatment should be resumed.

Screening Reduces Breast Cancer Mortality

Earlier detection of developing cancer should lead to increased cure rates and more effective treatments if the cancer is still localized. In an NCI-supported study, X-ray mammography used with physical examination in mass screening for detection of breast cancer has resulted in a one-third lower death rate from breast cancer over a five-year period. Dr. Phillip Strax reported results of the study, conducted by the Health Insurance Plan of Greater New York, in the *American Journal of Roentgenology, Radiation Therapy and Nuclear Medicine*.

Screening for breast cancer by X-ray mammography.

The study was the first controlled evaluation of a detection technique for cancer. Impetus for the study came from the realization that the annual death rate from breast cancer in the U.S. has remained unchanged at about 22 deaths per 100,000 women since the 1930's. Earlier detection offered a potential for lowering the death rate, because of the greater survival among women whose cancer is treated before spreading to the axillary (armpit) lymph nodes. Women with no axillary lymph node involvement have a five-year survival rate of 75 to 80 percent, whereas 50 to 55 percent of women with cancer in their lymph nodes at the time of detection are alive five years later. About half of the nearly 90,000 newly detected breast cancer cases in the U.S. annually have negative, cancer-free lymph nodes. The five-year survival rate for all breast cancer patients is about 65 percent.

In 1963, 62,000 women aged 40 to 64 were chosen at random from among participants in the Health Insurance Plan's medical groups. The women were chosen as 31,000 pairs with matched characteristics. In each pair, one woman was invited to participate in the study, to receive four annual clinical examinations and mammography. The other woman, as a control, continued to receive the usual comprehensive medical care at her medical group.

About 20,000 women (65 percent) in the study group responded to the invitation and received the initial screening examination. About 60 percent (12,000) of those screened initially completed the series of 4 annual examinations.

Breast cancer was found in about equal numbers in both the study and control groups: 296 breast cancers in the study group (including the women who were not screened) and 284 breast cancers in the control group. Over a five-year follow-up period, however, 65 deaths from breast cancer occurred in the control group compared with 40 in the study group—a difference of about one-third.

The scientists also calculated five-year fatality rates for the patients. The study group had a five-year fatality rate of 28 percent, one-third lower than the 42 percent rate for the control group.

When figures for the women who did not respond to the screening invitation were excluded, the effects of screening were even more apparent. The screening program detected 132 breast cancers among the 20,000 women who participated. The five-year fatality rate was only 17 percent for these cases. X-ray mammography detected 44 breast cancers (one-third of the 132 cases) before the cancers were detectable by physical examination. After 5 years, only one death from breast cancer had occurred among these 44 women—a fatality rate of 2 percent.

The scientists reported that 70 percent of the cases among the screened women had no axillary lymph node involvement. The rate of negative lymph nodes among the control group was 46 percent, indicating that more of the cancers had reached an advanced stage before being detected.

The scientists also observed that the reduced death rate occurred entirely among women 50 years of age and older. Deaths from breast cancer occurred at the same rate in both study and control groups among women aged 40 to 49 years. There was no apparent explanation for this difference.

Alpha-Fetoprotein in Cancer Diagnosis

Clinicians have long sought a blood or urine test specific for one or more forms of cancer. Dr. Thomas A. Waldemann and other NCI scientists have found that a substance called alpha-fetoprotein (AFP) occurs at elevated levels in the blood of patients with few diseases other than cancer.

AFP is associated particularly with cancers of the liver and testis. The scientists have developed a highly sensitive immunologic test for AFP that may be particularly useful in monitoring the effectiveness of therapy for these two forms of cancer. Dr. Waldmann presented results of clinical studies of the detection procedure at professional meetings.

The detection method, called double antibody radioimmunoassay, can measure concentrations of the cancer-related protein, AFP, at levels 1,000 times smaller than detectable with other tests usually used for the protein. Consequently, the method can detect cancer in patients who would have appeared cancer-free in other tests for AFP.

AFP is found at elevated levels in fetuses and in women during pregnancy. The concentration of AFP normally decreases after birth, to a level of less than 40 nanograms per milliliter of blood serum. (A nanogram is one billionth of a gram, a milliliter one thousandth of a liter). AFP levels are known to rise again, however, in some patients with liver cancer or cancer of the testis. Dr. Waldmann found that AFP levels were elevated in the serum of 95 of 130 patients (73 percent) with liver cancer, and in 63 of 79 patients (80 percent) with testicular cancer. Of the latter 63 patients, three-fourths had AFP levels between 40 and 3,000 nanograms per milliliter, the smallest concentration detectable by conventional tests.

The scientists have also been able to find elevated AFP levels in some patients with other types of cancer. They detected AFP levels above 40 nanograms per milliliter in 10 of 46 patients (22 percent) with pancreatic cancer, 17 of 100 patients (17 percent) with stomach cancer, 10 of 149 patients (7 percent) with lung cancer, and 9 of 189 patients (4.6 percent) with colon cancer.

Dr. Thomas W. Waldmann measures alpha-fetoprotein with radioimmunoassay.

Because AFP occurs at high levels in relatively few patients with cancer other than liver and testicular cancers, testing for this protein would probably not be useful in detecting the other cancers. Dr. Waldmann suggested, however, that other cancers may be detected more frequently if tests are made for several characteristic biochemicals, or "markers." For example, detection of gastrointestinal cancer can be improved by testing for both AFP and carcinoembryonic antigen (CEA). The NCI scientists were able to detect liver or testicular cancer in a greater percentage of patients by using the double antibody radioimmunoassay for human chorionic gonadotropin (HCG), as well as for AFP.

The highly sensitive test for AFP may find its most immediate application in providing physicians a measure of progress during cancer treatment. In studies of 15 patients to date, the scientists found that the level of AFP would drop rapidly after surgery, for example, but would not decrease below the 40 nanograms-per-milliliter level if some cancer cells

remained in the patient. If cancer cells remained and resumed their spread, the level of AFP began to rise again, indicating in advance of any other signs or tests the need for further treatment. The scientists also found that the likelihood of detecting a recurrence of cancer after treatment was increased by measuring two or more markers.

The use of AFP as a marker for some types of cancer is encouraged by the finding of relatively few "false positives"—cases in which other disease gave elevated levels of the protein.

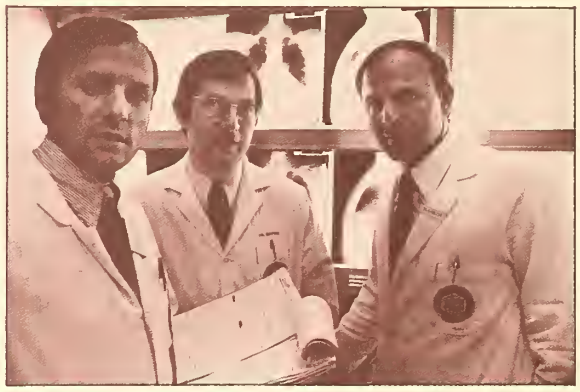
Combination Chemotherapy for Non-Hodgkin's Lymphoma

In April 1973 research physicians at NCI reported the first evidence that patients with various types of advanced non-Hodgkin's lymphoma can be rendered free from disease for up to seven years by the use of combination chemotherapy alone.

Eighty patients with non-Hodgkin's lymphomas were treated at the NIH Clinical Center between April 1965 and May 1972 using three basic treatment plans. The first drug protocol, called CVP, was a cyclical combination, repeated every 21 days, of cyclophosphamide daily for five days, vincristine on the first day, and prednisone daily for five days. If a complete remission (disappearance of all evidence of disease and return to normal performance status) was achieved after a minimum of four cycles, two additional "consolidation" cycles were administered. In those patients who failed to attain a complete remission, repeated cycles of the chemotherapy were given until there was either a complete disappearance of disease, or until regrowth of tumor occurred.

No maintenance drug therapy (continued administration) of drugs alone or in combination) was given to patients with documented complete remissions, with the exception of eight patients with lymphocytic lymphoma (a form of malignant lymphoma in which the predominating cell type belongs to the lymphocytic series) who achieved complete remission in 1970. This group of patients received maintenance cycles of CVP chemotherapy every 6 to 12 weeks.

The other two treatment plans were MOPP, consisting of nitrogen mustard on days 1 and 8, vincristine on the same days, procarbazine for 14 days and prednisone for the same number



Drs. Robert C. Young, Vincent T. DeVita, Jr., and George P. Canellos (l. to r.), clinicians who developed effective chemotherapy of advanced lymphomas.

of days, or a modification called C-MOPP, in which the drug cyclophosphamide was substituted for nitrogen mustard on days 1 and 8. Drugs were administered for two weeks out of each month for six consecutive months.

The study was performed by an NCI team headed by Dr. Vincent T. DeVita. Results were reported at a meeting of the American Society of Clinical Oncologists and the study was published in *Blood*.

"The results . . . with the CVP and MOPP programs in non-Hodgkin's lymphoma demonstrate that a significant proportion of all patients treated (28 percent) and 61 percent of those achieving a complete remission have had disease-free survival lasting from one year to more than seven years after termination of six months of combination chemotherapy," the scientists said.

In addition to achieving significant advances in the clinical treatment of these diseases, which were previously considered to be invariably fatal, the doctors demonstrated that detailed microscopic classification of the characteristics of each patient's cells and tissues was an important determinant in predicting the outcome of therapy and determining which protocol would be most effective. This finding correlates well with a recent large retrospective study of non-Hodgkin's lymphoma conducted at Stanford University with NCI support.

Several previous clinical studies outside of NCI demonstrated the superiority of combination chemotherapy in improving both remission rates and survival in non-Hodgkin's lymphoma compared to results previously obtained with single drugs. The NCI study re-

affirms the necessity of subdividing patients on the basis of detailed tissue studies. For example, it was found that one form of advanced disease, which traditionally has been quickly fatal, had a significant response to combination chemotherapy. There were 37 percent of the patients with this form, diffuse histocytic lymphoma, who completed 6 cycles of chemotherapy in complete remission; a finding of great importance is that none of these patients have had a recurrence of disease for periods of up to seven years after their last treatment.

These represent the longest survivals for this form of advanced lymphoma in the medical literature and the first proof that it may be cured or controlled for long periods of time by the use of drugs alone.

Of particular interest to future studies is the finding that patients in complete remission who relapsed did so exclusively in areas of previous disease involvement, with 69 percent of the relapses occurring in the lymph nodes alone. This raises the question as to whether the combined use of radiotherapy, and chemotherapy, as is presently under study with Hodgkin's disease at NCI and outside institutions, might lead to significantly longer disease-free survival. In theory, tumor cells which survive one form of therapy and retain the ability to cause a recurrence of disease might be effectively destroyed by adding another type of therapy.

Applanation tonometry measures intraocular pressure.

The Role of the Pigment Epithelium

Only within the past few years have vision researchers directed much attention to the single cell layer of the retina called the pigment epithelium. This narrow band lies between the sensory portion of the retina (the light-sensitive tissue at the back of the eye) and the underlying blood vessel-rich tissue, the choroid. Until recently, studies of retinal degenerative conditions usually were directed at the neural (sensory) portion of the retina.

In new research, however, investigators have discovered that the pigment epithelium apparently plays a vital role in the visual process, and efforts are now being made to explain this function both in normal retinal activity and in certain retinal diseases.

In an effort to clarify the functional relationship between the photoreceptor cells in the retina and the pigment epithelium, NEI-supported researchers headed by Dr. Kenneth Brown at the University of California, San Francisco, have identified a special anatomic relationship between the pigment epithelium and the neural portion of the cat retina.

Cones are one of two types of photoreceptor cells in the neural layer of the retina. They are specialized cells responsible for sharpness of vision and for color vision. The other type, rods, are responsible for night vision. It has been shown that the stalk-like outer segments of these photoreceptor cells project into the pigment epithelium, where they are provided a healthy *milieu*. In addition, the tips of the cell projections have a normal tendency to break off, and it is the healthy pigment epithelium which absorbs these materials. If this absorption does not occur, segments of the broken tips collect between the retina and the pigment epithelium, and this possibly leads to retinal degeneration.

Dr. Roy H. Steinberg, another member of the team, and his associates, have used both the scanning electron microscope and the transmission electron microscope to study the surface of the pigment epithelium which lies closest to the neural retina. They found that each cone's outer stalk is enclosed in a sheath arising from the surface of the pigment epithelium cell. The sheath, in turn, is formed by three or four separate leaf-like processes. This is the first time this specialized anatomic relationship has been positively identified and described in detail. Recently Drs. Hogan and Steinberg have shown in the human retina that these extensions of the pigment epithelium engulf portions of the cone outer segment, a function similar to that served by the pigment epithelium in the rod case.

For several years this group of investigators has also been studying the detailed functional relation between the neural retina and pigment epithelium.

This knowledge will contribute to understanding the factors underlying degenerative retinal diseases, the most frequent causes of blindness in this country. For example, it is already known that an abnormal pigment epithelium is directly involved in certain eye diseases for which there are now no cures. In macular degeneration, a deterioration of a small part of the retina responsible for fine or reading vision, the pigment layer is often missing or unevenly spaced. A breakdown in the normal interaction between the pigment epithelium and the photoreceptor cells may also underlie another retinal degenerative disorder, retinitis pigmentosa, which causes night blindness and gradual restriction of the visual field in children and adolescents.

Animal Model Developed for Glaucoma

While research has made it possible to treat medically most cases of chronic, open-angle glaucoma—the most common form—the ultimate goal is to find a means of preventing this leading cause of blindness. To approach this objective, researchers must better understand how glaucoma causes loss of vision. Progress toward this end has been delayed by the lack of an appropriate animal model for the disease.

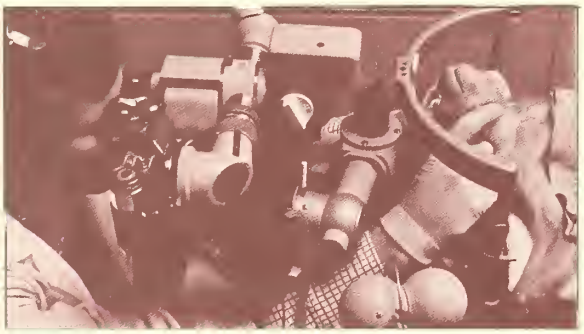
NEI scientists have now developed what appears to be the first suitable animal model for studying the effects of chronic elevation of pressure within the eye, a characteristic of glaucoma.

Drs. Douglas E. Gaasterland, of the NEI Clinical Branch, and Carl Kupfer, Institute Director, have developed a method for inducing changes in the eye of the rhesus monkey that closely resemble those observed in chronic, open-angle glaucoma in humans.

Glaucoma results when an obstruction in the outflow of a fluid, aqueous humor, leads to increased pressure within the eye, with sub-

sequent damage to the optic nerve and loss of vision. In chronic, open-angle glaucoma, although there is no anatomical evidence of blockage within the aqueous outflow channels, obstruction has been shown to exist by functional testing.

To produce sustained, elevated pressure, the researchers used argon laser photocoagulation. In this procedure an intense beam of fine, but brilliant, blue-green light is directed into the eye of the anesthetized monkey and aimed at



the aqueous outflow channels. The energy of the laser beam causes minute burns. Then the healing process creates an obstruction in the outflow channel.

Drs. Gaasterland and Kupfer report that repeated laser applications cause a sustained pressure elevation and a marked reduction in the outflow of the aqueous fluid from the eye. Retinal and optic nerve changes, similar to those seen in human chronic, open-angle glaucoma, develop.

The experimental ocular hypertension produced differs from previous models in several important ways. First, the sustained elevation of pressure is produced by the use of noninvasive technique. Second, the mild to moderate initial inflammation caused by the photocoagulation is temporary, lasting usually less than two weeks. Third, the primary alteration induced in the eye is localized in the region of the aqueous humor outflow pathways.

"This model provides us with a tool for studying why and how glaucoma causes visual loss," Dr. Gaasterland explained; "it allows us to conduct investigations that cannot be done in humans, but are necessary to our understanding of the effects of glaucoma in patients."

The investigators point out that there are a number of previous models of experimental, ocular hypertension. Each type has been associated with either a generalized damage to the eye or a pressure rise of insufficient duration to cause selective loss of retinal ganglion cells, a degenerative change that is required to make a diagnosis of glaucoma based on tissue studies.

Drs. Gaasterland and Kupfer conclude that the experimental ocular hypertension they have described is glaucoma because it results in cupping of the optic nervehead and the selective loss of retinal ganglion cells.

Further research will be needed to demonstrate conclusively the suitability of the monkey model. The investigators expect that their experimental method will significantly enhance research to understand better the basic processes underlying glaucoma and facilitate research directed toward finding means of curing or preventing it.

Instrument Developed for Vitreous Surgery

Until recently, opthalmologists have been severely limited in their ability to remove diseased vitreous from the eye. Despite the fact that pathologic changes in this clear, jelly-like substance are known to lead to serious ocular complications, such a procedure has usually been considered too risky to attempt. However, through the efforts of National Eye Institute-supported researchers, this situation is changing.

The vitreous body which fills the center of the eye can be affected by disease, aging, and injury. When the balance of fluid in the gel is disturbed, the vitreous liquefies and interrupts the visual process. In addition, retinal diseases such as diabetic retinopathy can damage the vitreous, causing visual loss.

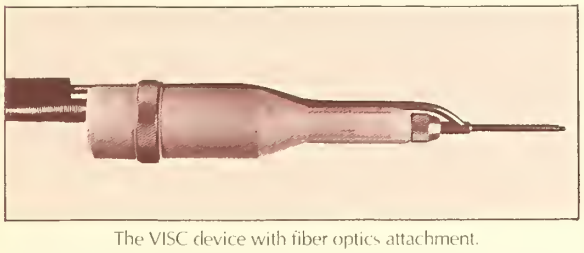
Previously, surgeons concerned about the effects of leaving diseased vitreous in the eye had been confronted with two serious problems. Attempts at vitreous removal have been made in conjunction with cataract surgery. However, because of the need to make a large incision in the cornea and the possibility of severe irritation of the iris, this technique has been considered hazardous.

Additionally, inadequate instrumentation hindered the surgeon in this technique. Available instruments were too large to work well, could not be easily introduced into the eye, and

obscured the surgeon's view. Furthermore, because the vitreous is responsible for maintaining the eye's shape, the organ could collapse during the operation. Despite these obstacles, some means for removing vitreous safely has long been sought.

What was needed was an instrument designed specifically to be small and delicate enough for this sensitive procedure. In response to this need, Dr. Robert Machemer and his associates at the University of Miami's Bascom Palmer Eye Institute began working with NEI support in the early 1970's on a device that would later be known as the vitreous infusion suction cutter (VISC).

Their motor-driven instrument is designed to enter the eye through the pars plana, an extremely narrow portion of the ciliary body adjacent to the retina. It therefore allows the cornea and lens to remain intact. The VISC permits the surgeon simultaneously to cut the vitreous, remove it from the eye, and replace it with a balanced salt solution. With this technique, the vitrectomy is performed under microscopic observation, and a contact lens is placed on the patient's eye, allowing magnification and stereoscopic control of the instrument. In addition, the VISC allows optimum illumination of the small operating area with the use of fiber optics which also are introduced into the eye.



The VISC device with fiber optics attachment.

Although the instrument is being evaluated and refinements are being made, it has already proved effective for treating a number of patients who were considered hopelessly blind from vitreal disease and retinal disease affecting the vitreous.

For example, Dr. Machemer has found the VISC useful in treating vitreous opacity due to hemorrhages which occur in injuries, vein occlusion, and Eales disease. He has also found that diabetic retinopathy patients with vitreous hemorrhages, but without proliferations of the retinal blood vessels, can be treated successfully with the instrument. Another condition amenable to the procedure is complicated retinal detachment. Here, Dr. Machemer has shown the VISC to be invaluable for removing the diseased vitreous so that the retina can be restored to its original position with a conventional reattachment procedure.

It is hoped that the VISC also may be useful in preventing abnormal blood vessel proliferation in severe retinal diseases.

Tracing Visual Pathways in the Brain

Fundamental studies of the complex structure and functions of the normal human visual system can lay the groundwork for clinical investigations into the cause, treatment and prevention of abnormal conditions or disease in the eye. Ambylopia, strabismus, nystagmus, and other sensory-motor disorders of the visual sytem are among the least understood vision problems. Diseases of the visual nervous system are generally not amenable to treatment or prevention; even diagnosis may be difficult. Nevertheless, information about the function of the visual nervous system may have a bearing on disorders affecting the nervous system in general.

When light falls on the retina at the back of the eye, a nerve impulse is generated which is transmitted by the optic nerve to a succession of structures in the brain, eventually arriving at a region called the visual cortex. The routes that these impulses travel are known as visual pathways, but the connections and interconnections of these routes have not been clearly delineated.

Now, an experimental technique developed by NEI-supported researchers is providing investigators with improved means for depicting the architecture of the pathways. Dr. W. Maxwell Cowan and his associates at the Washington University School of Medicine, St. Louis, have devised a method which allows identification of nerve cell processes, even at considerable distances from their origin. Their technique employs a cellular phenomenon known as axoplasmic transport, which is the flow of protein and other materials within a nerve cell. The protein carries information from the center of the cell, where it is synthesized, out to the axon, the essential conducting portion of the nerve fiber. Some protein accumulates at the end of the axon and so, by appropriately labeling the protein, it is possible to identify exactly where the nerve fibers terminate.

Previous methods for tracing nerve pathways through an extremely complex collection of nerve cells, such as that in the brain, have often been frustrated by the need to damage one part of the nervous system in order to identify degeneration in other parts. This approach usually resulted in the destruction, not only of the neurons under investigation, but also fibers passing through the region. In addition, the degeneration process inevitably altered the structure of the neurons being studied so that their natural anatomy could not be determined.

Dr. Cowan and his collaborators, however, have demonstrated that leucine and other amino acids labeled with radioactive tritium are readily taken up by nerve cells, incorporated into protein, and distributed at varying, but characteristic, rates to all the cell's processes. Only neurons whose cell bodies are close to the site of the isotope injection take up and incorporate the labeled material; nerve fibers passing through the site of injection are not labeled.

Using this method the precise origin of a neural pathway can be determined by identifying the number and distribution of the labeled cells. In addition, the technique permits the researcher to trace more clearly and accurately the course of the pathways and to identify precisely where they terminate.

Although this method has only been used with a limited number of protein precursors (amino acids), the technique should be useful in selectively labeling other transported materials similarly in order to trace their pathways, or in labeling differentially various celllular organelles that have characteristic distributions and functions within the neuron.

In this way, an experimental technique designed to provide better understanding of the visual system also may significantly enhance research to improve the prevention, diagnosis, and treatment of neurological diseases which affect human life.

Prevention of Cataracts

Cataract extraction is one of the most successful surgical procedures performed today. But cataract remains a leading cause of blindness in the United States, primarily because of social and economic factors. For this reason, cataract research is directed at finding a non-surgical treatment and a means of preventing this disorder.

A cataract is a cloudiness or opacity in the normally clear lens of the eye. It obstructs the passage of light to the retina, the eye's light-sensitive tissue, thus impairing vision. In most cases the cause of a cataract cannot be determined, but it is known that cataracts occur when there is a change in the chemical composition of the lens. Even though it is not necessary to delineate the exact cause of a cataract before surgically removing it, such knowledge is considered essential to finding a means of non-surgical treatment or prevention.

Dr. Jin H. Kinoshita, Chief of the NEI Laboratory of Vision Research, and his associates have been studying various types of cataract, and their research is increasing our understanding of cataract formation.

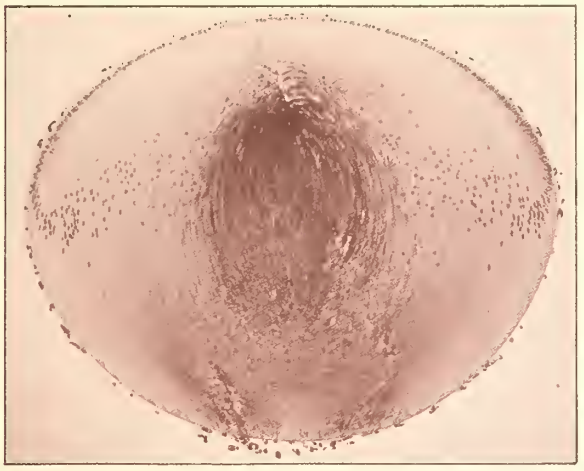
The investigators have found that osmotic swelling is a common feature of many cataracts. In the normal lens the state of hydration that maintains proper lens volume is controlled by two opposing forces. One is the permeability of the membrane enclosing the lens, and the other is a so-called pump mechanism that continually forces out sodium ions and concentrates potassium ions. These two factors are involved in osmosis in the lens; that is, the passage of various solvents through the lens membrane from a lesser to a greater concentration.

The fluids that surround and bathe the lens contain a high level of sodium and a low level of potassium. However, inside the lens there are high levels of potassium and low levels of sodium. If the osmotic process were to break down and the lens membrane were to allow a state of equilibrium to develop between these two elements, sodium would enter and potassium would leave the lens. This eventually would lead to a swelling of the lens tissue. Thus, maintaining the proper balance between the processes of extruding sodium and concentrating potassium is crucial to preserving the viability of the lens.

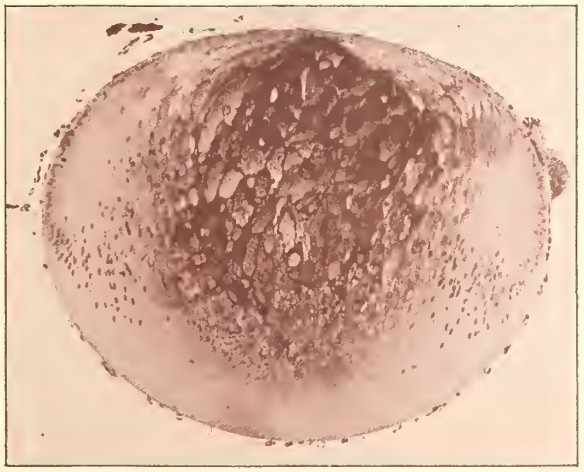
One form of cataract that Dr. Kinoshita and his associates have investigated is the sugar cataract, which may occur in diabetes and galactosemia. These sugar cataracts can be experimentally induced in animal lenses for laboratory study. The researchers have shown

that in both diabetes and galactosemia in experimental animals lens swelling is a constant feature of the cataractous process. The osmotic change has been shown to be due to sugar alcohols.

In the lens sugar is converted to sugar alcohol by the enzyme aldose reductase. Because the sugar alcohol is not further metabolized effectively and, therefore, does not penetrate the lens membrane readily, it accumulates to high levels once it is formed in the lens fibers. The



A normal, 18-day embryonic mouse lens (above) contrasted with a Fraser, 18-day embryonic mouse cataract.



higher osmotic pressure this creates is immediately corrected by an influx of water, causing swelling. The accumulation of sugar alcohol and lens swelling set into motion the cataract-forming process.

Dr. Kinoshita hypothesized that since aldose reductase appears to trigger the process, it might be possible to control its formation by inhibiting the enzyme. Through other research, a chemical that inhibits aldose reductase was identified. Using it, Dr. Kinoshita now has successfully prevented the formation of this type of cataract in animal lenses in test tube studies, and delayed its formation in the eyes of living animals.

This development led him to investigate another type of cataract, that associated with heredity. He directed his attention to a special strain of mice that normally develop a small lens opacity three weeks after birth. At the same time this opacity develops, Dr. Kinoshita observed, there is a sudden increase in lens hydration as well as a rise in the sodium level. He and his associates found that the failure to exclude sodium becomes more prominent as the cataract progresses.

Further research showed that activity of the enzyme Na-K ATPase (sodium-potassium adenosine triphosphate) is lower in the lenses of these cataractous mice. The Na-K ATPase is the key enzyme involved in maintaining lens hydration. The investigators also found that this enzyme is normal in the animal's other tissues. This had led Dr. Kinoshita to hypothesize that a cataract-inducing factor is present in the mice lenses. His studies revealed that a substance is indeed present in the cataract which inactivates Na-K ATPase.

If Dr. Kinoshita's theory proves correct, it may be possible to find a means of preventing inactivation of the enzyme and, thus, open the possibility of delaying the formation of this form or cataract or even preventing it.

Spurred by these findings, Dr. Kinoshita is also investigating the cause of the most common form of cataract, that associated with aging. He as well as other investigators—many working with NEI support—are concentrating efforts on determining what causes the so-called senile cataract. Through their research it may some day be possible by medical means to prevent or slow the development of this common cause of visual disability and blindness.

Coronary Care Unit substantially improves survival rates among hospitalized patients through advanced techniques for continuous monitoring, improved resuscitation procedures, and a more aggressive therapeutic approach to control complications.

Echocardiography in Cardiosvascular Diagnosis

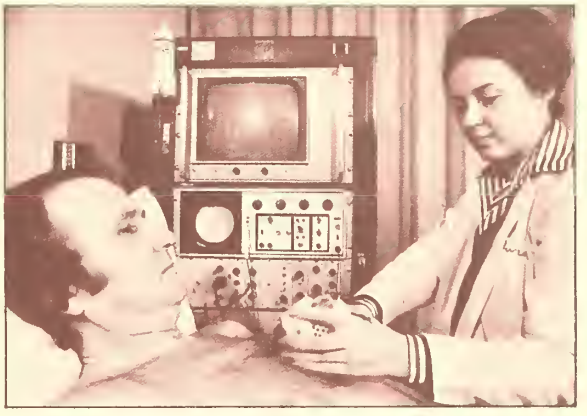
Echocardiography is a painless, safe, non-invasive technique for visualizing the heart and great vessels by means of reflected ultrasound waves. In particular, National Heart and Lung Institute studies show that two-dimensional echocardiography, an important advance over conventional one-dimension systems, is even more useful in the diagnosis and evaluation of several congenital and acquired heart conditions.

An oscillating transducer, or "scanner," is placed on the patient's chest and directs an ultrasonic beam through the intact chest wall to the section of the heart to be examined. Like X rays, ultrasound passes readily through many bodily tissues and fluids, but a portion of the beam is reflected from the surface of the heart and from the blood-tissue interfaces within it. The returning echoes are converted electronically into a cross-sectional image of the heart, permitting detection of structural defects and assessment of their effects on heart performance.

Two-dimensional echocardiography is highly effective in diagnosing defects such as transposition of the great vessels, tetralogy of Fallot, absence of a ventricular septum, and other congenital conditions resulting in cyanosis ("blue babies"). It often provides information on these conditions that cannot be obtained by conventional X-ray visualization (angiography) and, in some instances may even obviate the need for angiography, a procedure not without hazard in critically ill infants.

Both one and two-dimensional echocardiography are also uniquely valuable in the diagnosis of asymetrical septal hypertrophy (ASH), a genetically transmitted disease of heart muscle. In all cases of ASH, the ventricular septum (the wall between the ventricles) is thickened, especially in the vicinity of the aortic valve, through which all blood destined for the systemic circulation must pass. In some patients, this septal thickening may seriously impede the flow of blood through this valve, resulting in severe symptoms and eventually leading to congestive heart failure. In this obstructive form of ASH, however, the underlying heart-muscle abnormality tends to be confined to the ventricular septum itself, and surgical relief of the obstruction nearly always produces long-term improvement.

Non-obstructive ASH appears to be more common than obstructive ASH. And though non-obstructive ASH may produce no overt clinical symptoms or only very mild ones, patients are at increased risk of cardiac arrest or other serious events. In such instances the disease is more diffuse—frequently involving the whole left ventricle—and poses a definite threat of potentially fatal cardiac involvement.



Hand-held scanner (ultrasonic transmitter and receiver) for non-invasive two-dimensional echocardiographic visualization of tissue layers through a narrow cross-section of the heart.

Studies now in progress indicate that ASH may become clinically manifest in infancy, may be a factor in sudden infant death, or may lead to heart failure and death in the early months of life. It has become apparent that ASH may be more common than generally supposed, since non-obstructive ASH is often difficult to detect by techniques other than echocardiography.

Degenerative heart-muscle disease (cardiomyopathy) may occur as a consequence of other systemic disorders, including chronic alcoholism, amyloidosis, and several connective tissue or neuromuscular diseases. Such secondary cardiomyopathies are frequently difficult to detect by conventional clinical methods until the condition is well advanced. Echocardiography reveals heart changes due to cardiomyopathies before, and often long before, clinical signs become evident. It also provides a basis for classifying such cardiomyopathies; accurate classification is often useful in determining the best approach to treatment.

In addition to its value in cardiovascular diag-

nosis, echocardiography also has many useful applications in cardiac physiology. It was used, for example, in assessing the effects of prolonged space flight on heart performance in astronauts returning to earth after 85 days in Skylab III. Their long sojourn in space caused no deterioration in ventricular function.

The studies cited were done by Dr. Walter L. Henry and co-workers of the Cardiology Branch. Collaborating in the development of the equipment was Mr. James Griffith, of the Biomedical Engineering and Instrumentation Branch in the Division of Research Services.

Reduction of Heart-Attack Damage

Research conducted or supported by the National Heart and Lung Institute has yielded several promising methods for reducing the extent and severity of heart-muscle damage resulting from acute heart attacks.

Most heart attacks stem from drastically reduced or completely blocked blood-flow to the portion of the heart muscle normally serviced by an artery that is suddenly obstructed. The result is an area of blood-deprived, damaged, or dead tissue called an infarct.

The extent of irreversible heart-muscle damage following the acute attack is often a crucial factor affecting both survival and the amount of residual disability after recovery. This explains the Institute's interest in measures that may help to protect heart muscle against the consequences of blood deprivation (ischemia) and to salvage muscle not already hopelessly damaged during the very early stages of the attack.

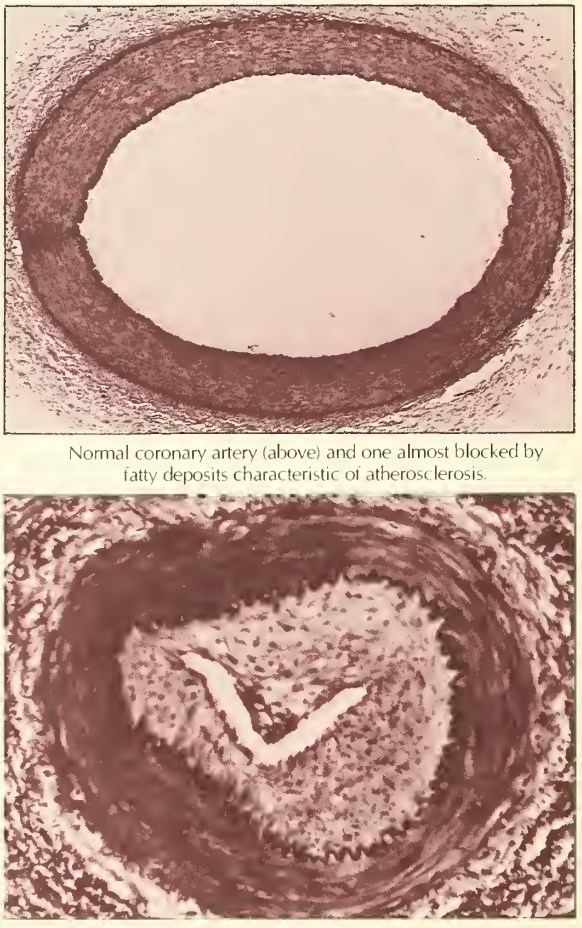
NHLI studies in animals indicate that nitroglycerin, the time-honored drug for relieving the chest pain of angina pectoris, may also be uniquely valuable in the treatment of acute heart attacks. In proper dosage, the drug reduces ischemic heart damage and also helps protect the heart against aberrations in heart rhythm, a common and sometimes lethal consequence of heart attacks.

Two effects of nitroglycerin had seemingly ruled it out in the treatment of acute heart attacks: the drug reduces blood pressure (and hence coronary artery perfusion pressure) and it triggers a reflex increase in heart rate. But the studies revealed that, if drug dosage were adjusted so that these effects were not excessive, nitroglycerin actually increased coronary bloodflow, reduced ischemic damage, and improved the electrical stability of the heart.

Moreover, the fall in blood pressure and increase in heart rate normally elicited by nitroglycerin could be abolished by giving the drug in combination with phenylephrine, a vasoconstrictor that enhanced the beneficial effects of nitroglycerin on heart muscle.

Clinical studies are now in progress to evaluate the effectiveness of nitroglycerin alone or nitroglycerin plus phenylephrine in reducing ischemic heart-muscle damage and other potential complications of acute heart attacks. The studies were done by Dr. E. M. Smith and associates of the Cardiology Branch.

Several research teams report that a circulatory-assist technique called balloon pumping reduces infarct size after an acute heart attack. The technique, developed by Dr. S. D. Moulopoulos, employs a balloon-tipped catheter that is threaded into the descending portion of the aorta, the large blood vessel carrying blood directly away from the heart. The balloon is



Normal coronary artery (above) and one almost blocked by fatty deposits characteristic of atherosclerosis.

then rhythmically inflated and deflated in synchronization with heart beat. Inflated as the heart refills between beats, the balloon helps to sustain systemic blood pressure and to improve bloodflow to the coronary arteries serving the heart muscle. Deflated as the heart contracts, it reduces the pressure that the left ventricle must overcome in pumping blood, thereby reducing its workload. The combination of increased coronary perfusion and reduced heart work appears responsible for an observed reduction of permanent heart-muscle damage.

Animal studies by several research teams, including those headed by Dr. Timothy J. Regan at the College of Medicine and Dentistry of New Jersey and Dr. Robert B. Jennings at Northwestern University, indicate that drugs called beta adrenergic blocking agents reduce the extent of ischemic injury after induced heart attacks. Propranolol and certain other drugs in this family reduce the workload of the heart without adversely affecting its blood supply. The doses used in the studies exceeded allowable human dosages, but work is in progress to determine whether these drugs, in smaller doses, might not still produce significant benefit in patients.

In animal and in clinical studies done at Peter Bent Brigham Hospital, Boston, Dr. Eugene Braunwald and co-workers have found that the enzyme hyaluronidase dramatically decreases heart-muscle damage occurring soon after acute heart attacks. The exact mechanism of action remains to be defined, but since the enzyme increases the permeability of susceptible tissues it is thought it may enable nutrient fluids to reach blood-deprived but still viable areas of heart muscle.

Studies by Dr. John Powell and co-workers at Massachusetts General Hospital and Dr. James T. Willerson at the University of Texas Southwestern Medical School, indicate that the drug mannitol also decreases infarct size. A potent osmotic diuretic, mannitol promotes the movement of fluid out of swollen cells. In the heart, it thus decreases cell swelling and may facilitate the movement of nutrient fluids into blood-deprived areas of muscle. It also improves heart-muscle contractility by increasing calcium availability.

Dr. Peter Libby and co-workers at Peter Bent Brigham Hospital, Boston, report that certain steroids may reduce heart-muscle damage after heart attacks, apparently by reducing inflammation and slowing the breakdown of tissues in ischemic areas.

These studies indicate that the usual destructive effects of acute heart attacks on the heart muscle can be minimized by timely and appropriate clinical interventions. They also suggest that, a more aggressive approach based on sound physiological and clinical principles might save many lives and restore many persons who would otherwise be permanently disabled to a normal existence.

Determination of Blood Lipids

Measurement of the blood level of two fatty substances, cholesterol and triglyceride, is extremely useful in estimating the risk of a patient's subsequently developing coronary heart disease. Precise measurements of these have proven difficult for many laboratories and clinics, however. It is insufficiently recognized that different methods employed for the determination of plasma cholesterol show wide variation in accuracy and considerable imprecision.

Chemist prepares electrophoretic cell to analyze lipoprotein patterns from patients' blood.

At the same time, large-scale screening of diverse populations for lipid disorders requires methods capable of handling a great number of samples in a reasonable amount of time. Manual testing techniques limit the number of patient samples that can be checked by one technician to approximately 50 per day. Automated methods now permit the testing of up to 80 samples in an hour.

The many variables in highly refined automated systems have previously made it difficult to standardize the machines to achieve the necessary degree of accuracy and precision.

The Laboratory Methods Committee of the NHLI Lipid Research Clinics Program, in cooperation with the Lipid Standardization Laboratory at the Center for Disease Control, Atlanta, have modified the Technicon Auto-Analyzer System and developed a stringent standardization procedure for these analyzers, which are in use at all of the 12 Lipid Research Clinics. This national standardization means that an identical plasma sample received at each of the 12 clinics will result in readings of cholesterol and triglyceride levels varying by no more than 3 percent. The standardization of the 12 instruments is maintained on a continuing basis. Two Lipid Research Clinics in Moscow and Leningrad are similarly standardized as part of a US-USSR collaborative research program.

This standardization has also been extended to other NHLI-sponsored collaborative studies, and Lipid Research Clinic Core Laboratories have acted as a focus for improving the performance of lipids determination in local community hospitals and commercial laboratories.

To facilitate the wider dissemination of standardized methods and protocols, the Lipid Research Clinic Program has made available the *Lipid Research Clinic Laboratory Method Manual*. The manual has already been translated into Russian and Japanese, and is presently in use by researcers in Israel, Sweden, Scotland, Germany, Japan and Russia.

Hopefully these combined efforts will lead to national and international agreement for the highly precise determination of these important blood lipid risk factors.

Molecular Basis for Familial Hypercholesterolemia

A related research effort concerning elevated blood lipids has further extended understanding of one of the five forms of lipid-transport disorders known collectively as hyperlipoproteinemia.

Though an important source of cholesterol is in dietary intake, the principal producer of the circulating cholesterol in human blood plasma is the liver. The synthesis of cholesterol involves a number of steps, one of which is controlled by the enzyme, HMG CoA reductase. The liver binds cholesterol and other fats to a protein, forming what is known as very low density lipoprotein (VLDL).

VLDL is composed of 12 percent cholesterol, 10 percent protein, 60 percent triglyceride, and 18 percent phospholipids. By metabolic pathways which have yet to be fully elucidated, VLDL is reduced to low density lipoprotein (LDL). LDL is composed of 50 percent cholesterol, 25 percent protein, 10 percent triglycerides, and 15 percent phospholipids.

Increased plasma level of LDL characterizes Type II hyperlipoproteinemia. This disorder may occur as the result of a secondary cause, such as hypothyroidism, but often it is the result of a primary, genetically determined abnormality. Previous studies of primary familial Type II hyperlipoproteinemia, or as it is also known, familial hypercholesterolemia, strongly suggest that it is inherited as a dominant genetic trait, much as brown eyes are dominant over blue eyes.

Familial Type II hyperlipoproteinemia may in fact be one of the commonest genetically determined abnormalities and is an important subject of study because of its involvement in increased risk of coronary heart disease (CHD). For example, homozygotes (persons inheriting two defective genes for the disorder) may die as early as 18 months, and rarely live past 20 years, due to the effects of the complications of CHD. Heterozygotes (inheriting one defective gene and one normal one) also have a much higher risk of CHD, usually manifesting symptoms before 50 years of age, though sometimes as early as their 20's or 30's. Most heterozygotes can lower their LDL levels to near normal with proper low-cholesterol diet and drug therapy, but little is known about the precise biochemical defects underlying the disorder.

It was demonstrated in the NHLI laboratories in Bethesda a few years ago that the increased levels of LDL in the blood of Type II patients resulted from a decrease in LDL clearance or removal from the bloodstream.

Dr. Joseph Goldstein and his associates at the University of Texas Southwestern Medical School in Dallas have now further clarified this clearance defect. In research funded by the NHLI, Dr. Goldstein grew tissue cultures derived from the skin cells, or fibroblasts, of normal, heterozygote, and homozygote patients. The use of skin fibroblasts to study biochemical processes is based upon the observation that many body cells, including those of the skin, possess the ability to act as "miniature

livers" in metabolizing fat.

The Dallas researchers observed that in normal human fibroblasts the rate of cholesterol synthesis is regulated by the presence of LDL in the culture medium. In order to suppress cholesterol synthesis, LDL is first bound to specific receptors on the cell surface, thus leading to the inhibition of HMG CoA reductase activity; HMG CoA is the important enzyme involved in cholesterol synthesis.

Dr. Goldstein's group observed that presumed homozygotes for familial hypercholesterolemia were almost completely deficient in the cell surface receptors for LDL and thus were unable to bind the lipoprotein. Consequently HMG CoA reductase activity was not suppressed and cholesterol was overproduced. In addition, the inability to bind LDL seemed to reduce LDL breakdown, another factor increasing its concentration in the blood plasma.

Heterozygotes showed a 60 percent reduction in LDL cell surface receptors and showed corresponding, but less severe, overproduction of cholesterol and diminished LDL breakdown. This approximates one half of normal LDL binding capability and lends further support to the concept of a dominant genetic mode of transmission for this disorder.

These findings should prove of worth, both by contributing to the understanding of a common and important human disorder, and by providing a new laboratory system for studying genetic phenomena.

Continuous Positive Airway Pressure in "Hyaline Membrane Disease"

Continuous positive airway pressure (CPAP) as a treatment for respiratory distress in newborn children now yields survival rates in the range of 90 percent. This amounts to a clear therapeutic breakthrough against this leading cause of neonatal death.

In about one percent of all newborn babies, life is immediately threatened because their lungs are immature and unable to retain the first breath of air. The first breath taken by all babies requires a great struggle, calling for 10 or 15 times the pressure needed to continue breathing once the lungs have been normally inflated. But immature lungs cannot retain the air; their air spaces collapse when the first breath is exhaled, and each succeeding inhalation is a struggle like the first. As areas of persistent lung collapse, or atelectasis, begin to spread and the infant's exhaustion and asphyxiation increase, plasma may ooze out into the repeatedly collapsing airways and form the glistening lining which formerly prompted physicians to name the syndrome "hyaline membrane disease." Now regarded as a condition of lung immaturity and largely an affliction of the prematurely born, the respiratory distress syndrome affects about 40,000 newborns in the U.S. each year and, until recently, killed more than half of these.

The idea of keeping the airways of these infants under continuous positive pressure originated with Dr. George A. Gregory and his associates at the University of California School of Medicine in San Francisco. CPAP therapy was conceived as a way of overcoming an abnormally high surface tension in the lungs. Evidence had long been accumulating that the lungs of infants with hyaline membrane disease are deficient in a complex surface-active substance that normally lines the air spaces at birth and regulates their surface tension during the breathing cycle. As inhalation enlarges the area of lung surface in contact with the air, elastic properties in this "surfactant" coating the airways come progressively into play, increasing airway surface tension and enhancing the lung's elastic recoil for the next, expiratory phase of the cycle. Then, as expiration rapidly shrinks the lung surface area, the surfactant sharply reduces the amount of surface tension that must be overcome to start the next inhalation. Without the surfactant to stabilize them, the immature lungs are forced to collapse on each expiration by the high surface tension.

"We thought that the application of a continuous airway pressure sufficient to balance the high surface tension might overcome the atelectasis and improve the arterial oxygenation," Dr. Gregory explained in the *New England Journal of Medicine* in which he and his collegaues described their therapeutic successes.

At first, the team applied the airway pressure through a tube in the infant's trachea, but they came to prefer a small pressure chamber over the head only, like a tiny diving helmet, which has since become widely known as the "Gregory box." With either approach, humidified oxygen or high-oxygen air is forced in continuously at slighlty elevated pressures (up

to 14 millimeters of mercury). The Gregory box is also equipped with an anesthesia bag to permit artifically assisted breathing if necessary. With CPAP alone, the infant breathes spontaneously.

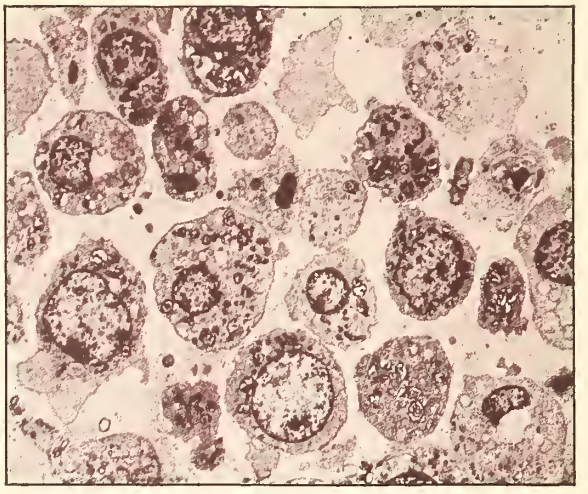
The effect on most of the struggling infants is dramatic and immediate, with relief obvious. Holding the collapsed lungs steadily open, CPAP extends the lung surface area so desperately needed to oxygenate the blood, and the measure of oxygen tension in the blood (PO_2) soars toward normal, often doubling in minutes. CPAP therapy is technically complicated, professionally challenging, and costly—a nurse for each baby is needed, according to some authorities. However, if CPAP and associated artificial ventilation can be sustained long enough (a week or so is about average, although a month or more is possible) most newborns with the syndrome gradually acquire the ability to breathe normally. For Dr. Gregory and his co-authors in San Francisco, the success rate now exceeds 90 percent—a figure unimaginable ten years ago.

Dr. Gregory's research was supported by grants from the NHLI, NIGMS and NICHD.

Surfactant-Producing Cells Available for Study

The problem of respiratory distress in the newborn is rooted in their lack of a pulmonary surfactant necessary for normal lung expansion. To fully understand the role of this surfactant, more fundamental knowledge of the cell biology of the lung is needed. Drs. Yutaka Kikkawa and Kokichi Yoneda of Albert Einstein College of Medicine in New York City have developed a method of lung cell isolation that makes homogenous populations of granular pneumocytes available for the first time. This advance is important because the granular pneumocyte—one of more than forty different types of lung cells—is the source of the surfactant missing in these babies.

By 1973 good methods were available for dispersing lung tissue into heterogeneous populations of individually viable cells, but in such mixed populations the value of biochemical studies is limited because of the effects which the secretions of different cell types exert upon one another in the same medium. Isolation of a homogenous cell population is a first step toward understanding the complex events in pulmonary biology. Drs. Kikkawa and Yoneda



Electron microscopy (x 1,500) reveals nearly homogenous population of granular pneumocytes isolated from the lung and cultured for studies of surfactant production.

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reported their new method in *Laboratory Investigation;* their research was financially aided by the NHLI.

The respiratory distress syndrome still accounts for the largest portion of infants who die in our nurseries. It is already clear that the study and biochemical manipulation of granular pneumocyte metabolism can help explain why these babies lack surfactant, and can also provide clinically useful ways of stimulating its development in infants with the syndrome.

Biocompatible Materials Development

Advances in the development of biocompatible materials for use in blood oxygenators, heart-assist devices, artificial heart valves, and other cardiovascular prostheses have resulted from recent research.

Blood Oxygenator Membranes

Blood oxygenators, the "lungs" of heart-lung machines used in open heart surgery, also hold considerable promise in the treatment of acute respiratory failure. Respiratory failure may result from severe lung infections, or may develop as a complication of emphysema and other chronic lung diseases. It is a common cause of death after surgery or severe trauma.

The most promising type of oxygenator for this purpose is the membrane oxygenator, in which blood flowing through the device is shielded from direct contact with the oxygen stream by a thin membrane. The membrane is permeable to both oxygen and carbon dioxide,

and gas exchange occurs across it in much the same manner as in the normal lung. The gentle blood-handling properties of membrane oxygenators of recent design enable them to provide respiratory assistance for prolonged periods—up to a week or more in several instances—until the patient's lungs have recovered sufficiently to resume normal function.

NHLI scientists have developed casting techniques that eliminate the chief cause of membrane failure in such oxygenators, pinhole defects in membranes prepared by conventional techniques. The scientists report casting silicone rubber membranes less than 1/1000 of an inch thick that are free of defects. The defect-free silicone membranes have been incorporated into a spiral-coil membrane oxygenator developed at NHLI. In animal experiments the membranes exhibited excellent gas permeability and blood compatibility characteristics.

The aim of this research by Dr. Theodor Kolobow and associates is to develop an oxygenator that can safely provide respiratory assistance for as long as a month—long enough for almost any acute respiratory emergency to have run its course.

Two other promising blood-compatible membrane materials have been developed by Drs. J. R. Petersen and L. T. Rozelle of the North Star Research and Development Institute, Minneapolis, and by Dr. J. M. Ketteringham of A. D. Little, Inc., Cambridge, Mass., under research contract support of NHLI's Biomaterials Program. Membranes fashioned from the new materials possess superior gas-exchange properties and enhanced biocompatibility compared to other known compounds.

The Kolobow spiral coil membrane lung.

Biocompatible Materials for Cardiovascular Prostheses

Blood compatibility is a prime consideration in selecting a material for any cardiovascular prosthesis that is in prolonged contact with blood. It sometimes happens that a material otherwise suitable for a prosthesis is incompatible with blood, or that a material with excellent blood compatibility may not have the prerequisites of durability, ease of fabrication, or mechanical characteristics desirable for some particular device.

This is the case with hydrogels, a class of gelatinous compounds with excellent blood compatibility, but lacking mechanical strength and toughness. NHLI contract-supported investigators have developed techniques for bonding thin coatings of hydrogels to the surfaces of a number of polymers, excellent in other respects for prostheses whose only drawback is that they play havoc with blood after prolonged contact. The result has been a group of the most promising materials yet developed for the fabrication of certain prostheses. These techniques were the work of Drs. Paul L. Kronick of the Franklin Institute Research Laboratories, Philadelphia, D. David Halpern of Polysciences, Inc., Warrington, Pa., and G. T. Kwiatkowski of Union Carbide Corporation, Bound Brook, N.J., following an initial suggestion of NHLI's Dr. Stephen D. Bruck.

In a related development from NHLI's Biomaterials Program, artificial blood vessles with mechanical properties similar to living tissue have been fabricated by Dr. J. L. Kardos of Washington University, St. Louis. Among their advantages are resistance to buckling or kinking when flexed (such as would occur in a prosthetic vessel traversing the region of the knee, for example), and normal response to blood pressure changes in propagating pressure pulse waves generated by the heart. Similar materials may find application in artificial heart valves and other prostheses.

Laboratory Replication of Inherited Molecular Diseases

National Heart and Lung Institute scientists have developed a cell-free system for protein synthesis, and with it have studied human genetic defects in the test tube.

In experiments by Drs. W. French Anderson and Arthur W. Nienhuis and colleagues, portions of the genetic code operant in thalas-

semia (Cooley's anemia) and sickle cell anemia were "read" by the subcellular genetic translation machinery of rabbit red blood cells to produce the abnormal hemoglobin patterns characteristic of the human diseases.

The reproduction of the molecular defects at the subcellular level has improved understanding of these diseases, and further manipulation may lead to effective means of therapy and control. The work, in addition, eventually may allow scientists to make a biochemical inspection of the protein-synthesizing machinery in other kinds of human disease.

The subcellular components used in the studies were isolated from immature red blood cells called reticulocytes. This kind of cell proved ideal, first because it is essentially a sac containing only the cell's genetic translation machinery, and second because it manufactures primarily one end product—the protein hemoglobin.

Reticulocytes from human thalassemia and sickle cell anemia patients yielded messenger RNA (mRNA), nucleic acid molecules that carry coded "blueprints" for hemoglobin synthesis. Other components of the genetic translation machinery were extracted from rabbit reticulocytes: 1) ribosomes, cellular structures that serve as a workbench on which the hemoglobin is put together according to the mRNA blueprints; 2) elongation factors, responsible for "decoding" the mRNA blueprint; 3) amino acids, the raw materials of protein which, when linked end to end in a precise sequence, form hemoglobin; 4) transfer RNA (tRNA), another type of nucleic acid molecule that carries (or "transfers") the amino acids from the cell "sap" to the ribosomes, and 5) at least three initiation factors essential for triggering the manufacture of new hemoglobin.

Incubation of all these components resulted in the production of hemoglobin chains identical to those in the corresponding disease state; that is, the coded instructions in mRNA isolated from thalassemic reticulocytes were faithfully translated into hemoglobin chains of predominantly the alpha type characteristic of thalassemic hemoglobin. Similarly, reticulocyte mRNA from sickle cell anemia patients engendered in the rabbit cell-free system the human alpha and beta S chains of sickle cell hemoglobin.

Separate but simultaneous runs, one involving the incubation of isolated mRNA as just outlined, the other using intact human reticulocytes, confirmed that thalassemic and sickle cell mRNA are able to duplicate exactly the hemoglobin-synthesizing abnormality present in intact bone marrow cells and reticulocytes. This result is consistent with the view that the molecular defect in hemoglobin in each of these inherited blood diseases is due to a quantitative or qualitative defect in the messenger RNA that directs hemoglobin synthesis.

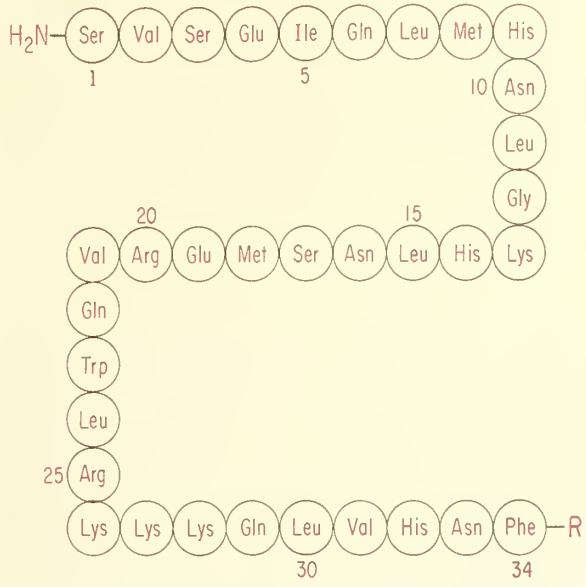
Human Parathyroid Hormone Characterized, Synthesized

Scientists at the National Heart and Lung Institute, the Mayo Clinic, and the Ciba-Geigy Pharmaceutical Company have succeeded in determining the chemical structure and synthesizing the biologically active portion of human parathyroid hormone.

These collaborative efforts revealed significant differences between the chemical structure of human parathyroid hormone and that derived from animal sources. They also make possible, for the first time, synthesis of sufficient quantities of the hormone's active component for experimental studies of its role in calcium metabolism and metabolic bone disease, for the development of diagnostic assay procedures for its measurement in human blood, and for clinical investigation of its potential use as a therapeutic agent in human disease.

Details of the chemical structure, including the precise sequence of the amino acids in the active portion of the human parathyroid hormone, were reported by Dr. H. Bryan Brewer, Jr., and colleagues of the National Heart and Lung Institute; and by Dr. Claude D. Arnaud and associates of the Mayo Medical School and Clinic, Rochester, Minn. This structural information was provided to a research team at Ciba-Geigy, Ltd., in Basel, Switzerland, which synthesized the single-chain molecular fragment by stringing together the constituent amino acids in the proper sequence and conducted animal tests to confirm the biological activity of this man-made peptide. The Ciba-Geigy team was headed by Dr. Werner Rittel.

Parathyroid hormone is normally present in such small amounts (fresh parathyroid gland tissue contains only 50 parts per million of the hormone), that hormone-secreting tumors obtained at surgery were used as the source of hormone for these studies. Yet, so uncommon



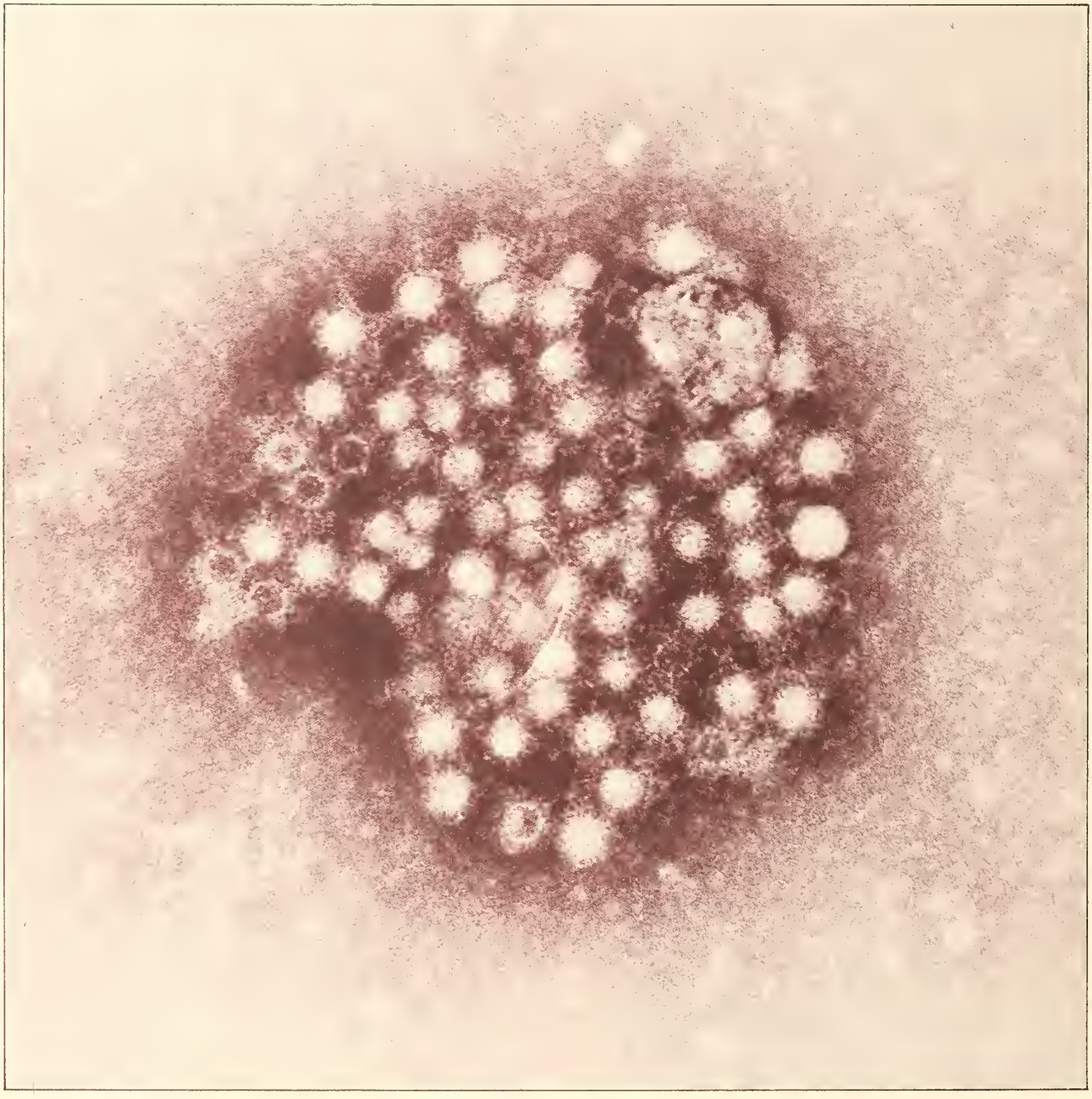
Molecular "blueprint" of 34 amino acid constituents of the biologically active portion of human parathyroid hormone.

are these tumors, that two years time and the cooperation of more than 150 institutions and individual physicians and surgeons in 12 countries were required to obtain the hundreds of frozen tumors needed for an adequate yield of hormone. These glands were collected world-wide by Dr. Arnaud and colleagues.

Active purified fractions from extracts of these glands were prepared by the Mayo Clinic team, from which Dr. Brewer and colleagues at the NHLI were able to isolate 3.8 milligrams of the pure human hormone—enough to determine the amino acid sequence of the biologically active region of the hormone (the first 34 of the 84 amino acids comprising the molecule).

Sequence determination was accomplished by subjecting the isolated hormone to sequential degradations by the Edman technique, whereby one amino acid at a time is "cleaved" off the chain, and identified by either gas chromatography or mass spectrometry. The sequential degradations were performed automatically in the Beckman Sequencer, a machine that carves amino acids off the parent molecule at a rate of one amino acid every two hours. The amino acid sequence of the biologically active portion of the human parathyroid hormone differed from the corresponding sequence of the bovine molecule by 5 and from the porcine hormone by 4 amino acid residues.

Virus-like particles revealed by immune electron microscopy in specimen from hepatitis A patients. (x 112,500).



Virus Causing Hepatitis Identified

Infectious diseases are as old as man and the microorganisms causing them even older. Some scientists believe that when life first sprang from the non-living chemicals which enriched primeval pools, it took the form of primitive bacteria.

Man's long association with microorganisms has not yet resulted in complete knowledge of these sometimes troublesome bits of living matter nor in complete control of the infections they produce.

The pursuit of such agents is an important goal of the National Institute of Allergy and Infectious Diseases (NIAID). Scientists engaged in this pursuit are equipped with tools and knowledge growing out of many scientific disciplines, such as virology, bacteriology, chemistry, and immunology. And, though the focus of NIAID's efforts is on conquest of disease, the Institute assumes responsibility for continued support of research in the basic sciences, knowing that progress in these fields leads to advances against a variety of human afflictions.

For example, last year, NIH-supported scientists, using sophisticated scientific tools forged in basic research laboratories, closed in on the agents believed to cause viral hepatitis.

In the United States alone, more than 60,000 cases of this debilitating and sometimes fatal disease are reported annually, and the true incidence is believed to be 10 times this figure.

Two forms of viral hepatitis—each caused by a different agent or agents—are recognized: hepatitis A (infectious hepatitis), and hepatitis B (serum hepatitis). The two forms produce almost identical symtoms—fever, malaise, nausea, and jaundice—but differ in incubation period and usual mode of transmission. Hepatitis A develops within 2 to 6 weeks of exposure and hepatitis B has a longer incubation period (6 weeks to 6 months).

Hepatitis A is believed to be the culprit in most large community-wide outbreaks. The agent appears to be carried most often in the stools of infected individuals and to be spread by contaminated food or water.

It is this agent which NIAID scientists now believe they have visualized through use of the technique of immune electronmiscroscopy (IEM). The organism, which Drs. Stephen M. Feinstone and colleagues captured on film during the past year was found in the stools of persons experimentally infected with infectious hepatitis. The stool specimens and sera were collected several years ago by other investigators conducting studies of hepatitis in human volunteers.

IEM involves the use of blood sera from convalescent patients. These sera usually contain antibodies (protective substances) specific for a causative organism. When mixed with material carrying the organism, antibodies in the sera coat the infective particles and make them easier for the electron microscopist to see. IEM is particularly useful in specific identification of a very small virus which may be present in a specimen in small numbers.

Such was the case with the particle found in hepatitis A stool specimens. It resembled members of the picorna and parvovirus families, and was found only in acute illness specimens and not in those obtained before infection. As further proof of the particle's causative role, antibodies to the suspect agent were detected in sera obtained from persons who had recovered from hepatitis acquired naturally or experimentally.

The investigators believe their data strongly suggest that the particle they observed is the causative agent of hepatitis A. Electron microscopic identification of the virus should help scientists isolate it in cell culture and grow it in quantities necessary for vaccine development.

Just as exciting as the hepatitis A discoveries were last year's findings on hepatitis B. Research on serum hepatitis has been brisk for more than 10 years but definitive identification of the causative virus has been elusive. In November 1973, however, a team of NIH-supported scientists reported that they had found evidence that the agent causing serum hepatitis is a virus and is probably the so-called Dane particle, one form of the hepatitis B antigen (Australia antigen) long associated with hepatitis B disease.

The evidence obtained was indirect, in that what the scientists actually found was a marker of viral activity—an enzyme—associated with the Dane particle. The scientists at Stanford University School of Medicine, Palo Alto, Calif., and at NIH who made this discovery worked with blood serum samples obtained from chronic carriers of the hepatitis B antigen. These sera had been concentrated and purified

by Dr. John L. Gerin of the Molecular Anatomy Program (MAN) Laboratory, sponsored jointly by NIAID and the Atomic Energy Commission (AEC).

In each of 8 hepatitis B antigen-rich sera, the enzyme (called DNA polymerase) was detected. This enzyme, which directs the synthesis of deoxyribonucleic acid (DNA), one type of viral genetic material, could not be found in any of 7 sera samples from people who did not have hepatitis B antigen.

From other experiments, the investigators determined that the core of the Dane particle most likely contains a DNA "template" that directs the synthesis of viral DNA. They believe that their data indicate that the Dane particle is the long-sought human hepatitis B agent and that it is a DNA virus. Again, as in hepatitis A, identification of the causative agent should spur the development of preventive measures.

In addition to NIAID and AEC support, these scientists received funds from the National Cancer Institute, the National Institute of Arthritis, Metabolism, and Digestive Diseases, and the Damon Runyon Fund.

Mouse Leukemia Viruses Found Inherited

Other viral research within NIAID (partly supported by the National Cancer Institute) is focused on the problem of viruses causing cancer. Here again, very sophisticated research procedures, developed in the process of carrying out fundamental studies of viral genetics, are enabling scientists to come closer to full understanding of how and why some cancer develops in laboratory animals. It is hoped, of course, that this knowledge can eventually be applied to the problem of cancer in man.

About 3 years ago, Dr. Wallace Rowe and his co-workers found that treatment of mouse embryo cell cultures with either of two specific chemicals consistently and rapidly caused the appearance of a mouse leukemia virus where none was formerly evident. The cells used in the cultures came from a strain of laboratory mice (AKR) known to have a high incidence of viral leukemia. These studies indicated that, even in seemingly normal cells of certain strains of mice, instructions for a leukemia virus may be present in unexpressed form as part of the cell's DNA, or blueprint, and may be inherited in the genetic process.

Work on the presence or lack of these instructions in cells was accelerated during the past year through development of a method for measuring the amount of viral genetic information found in the DNA of mouse cells. The procedure involves use of a chemical probe—a single-stranded DNA complementary to the RNA, or genetic material, of the mouse leukemia virus.

Using this probe, Dr. Rowe and his associates have found that the complete leukemia virus genome is present in the DNA of cells from the high-leukemia AKR mice. In contrast, only two-thirds of the virus genetic information was found in cells from another strain (NIH) of mice in which leukemia rarely occurs. The investigators believe that the fact that the DNA of NIH mouse cells lacks a significant portion of the mouse leukemia virus genome may explain why scientists have been unable to detect or to activate an endogenous leukemia virus (one originating within the organism) from this strain of mice.

Since the techniques employed in these studies make it possible to differentiate between DNAs of high-virus and truly virus-negative strains of mice, it is now possible for scientists to explore the basis of endogenous virus expression in other mouse strains and perhap in man.

Flu Viruses May Be Barnyard-Bred

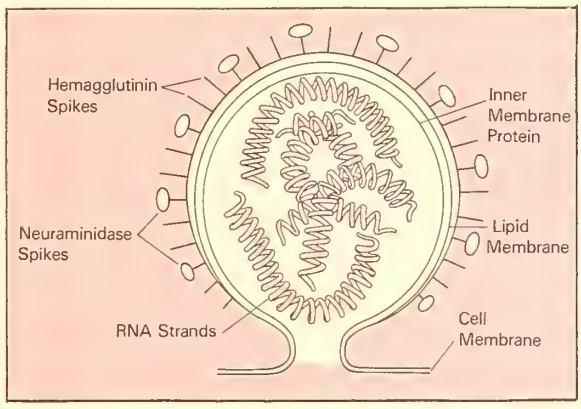
The ability to study various aspects of viral activity is also beginning to unravel some of the mystery that has long surrounded the virus causing influenza. This disease continues to be one of man's most baffling health problems, largely because the influenza virus undergoes extreme variation as it multiplies, and this necessitates constant up-dating of an effective vaccine.

Each year the influenza A virus—the type most frequently causing human disease—shifts slightly. This is thought to be due to minor selective mutations occurring as the virus meets a more and more immune population and adapts by changing its antigen pattern in order to survive.

Of more concern is a major change in the virus which seems to take place about every 10 years. At these times an essentially new virus appears, to which the population has little or no immunity. These "new" viruses cause the great pandemics (world-wide epidemics) of

influenza such as the one occurring in 1968-69 with the Hong Kong flu.

Research by an NIAID grantee, Dr. R. G. Webster, and his associates at St. Jude Children's Research Hospital, Memphis, has recently thrown light on the origin of these pandemic viruses, and provided evidence that they are recombinant viruses formed as the result of mixed infection of a barnyard creature (such as a horse, a duck, or a pig) with human and non-human virus strains.



Schematic of influenza virus showing hemagglutinin and neuraminidase sub-units on surface.

This evidence is based on development of sera specific for each of two chemical substances found on the surface of the influenza virus. These virus subunits are called hemagglutinin and neuraminidase. Antibodies against these subunits are formed in the blood following natural infection or immunization.

When the "new" Hong Kong virus appeared, scientists, using sera containing antibodies specific for these antigenic subunits, found that, though the neuraminidase of the Hong Kong strain was immunologically related to previous strains, the hemagglutinin was entirely different. In fact, further work by Dr. Webster and his associates showed that this "new" hemagglutinin was closely related to hemagglutinin subunits of two strains of influenza virus isolated from horses and ducks in 1963—5 years before the Hong Kong strain appeared. Analysis of the chemical structure of the hemagglutinins of the three strains (Hong Kong, duck, and horse) revealed even more similarities, and the Memphis investigators now speculate that the three viruses arose, by genetic recombination, from a common ancestor.

Such recombination, resulting in antigenic hybrids, has been possible in the laboratory for some time. Scientists need only infect a cell culture with two strains of virus to produce a number of different combinations. Recent experiments have also shown that antigenic hybrids can be isolated from animals and birds after the mixed infection of pigs, turkeys, and chickens with type A influenza viruses of porcine and avian origin.

The high yield of recombinant influenza viruses from mixed infections is probably due to the structure of the virus genome, or blueprint, which—unlike most viruses—is known to consist of at least six discrete segments.

Dr. Webster believes that all these pieces of evidence, taken together, lend weight to the hypothesis that "new" strains of human influenza viruses arise by genetic interaction between strains of human and animal (or avian) origin. The immediate practical application of Dr. Webster's research has been development of techniques for identifying surface antigens of the virus; a new type of influenza vaccine is now under development against the neuraminidase alone. Of even more importance for the future is the possible identification of influenza viruses in animal hosts that may serve as the source of pandemic viruses for man.

Encephalitis Viruses Survive in Mosquito Eggs

In the field of viruses transmitted by insects, one of the most important findings last year was the demonstration by NIAID grantees at the University of Wisconsin that the LaCrosse (LAC) encephalitis virus is passed from one generation of mosquitoes to the next in the female's eggs. This mechanism—never before demonstrated in a mosquito-borne virus—probably explains the virus' ability to survive freezing winters.

LAC virus has plagued parts of rural Wisconsin for about 20 years, attacking young children primarily and causing severe illness. Cases occur each year and are especially numerous following wet summers.

Earlier studies indicated that the LAC virus is maintained in nature by a mosquito-small mammal cycle, with the *Aedes triseriatus* mosquito acting as the principal agent of virus spread. What was not known was how the LAC encephalitis virus manages to survive during severe Wisconsin winters when adult mosquitoes are inactive.

Suspecting that mosquito larvae might be involved, Drs. Thomas H. Yuill and Wayne H.

Thompson looked for virus in eggs produced by 41 experimentally infected mosquitoes and in larvae and adults developing from the eggs. They found that the eggs contained virus, but in low concentration. However, larvae hatching from the eggs contained more virus, indicating that the agent had multiplied during larval development. Adults developing from the larvae also proved to be infected.

New knowledge of LAC virus gained in this study will undoubtedly help efforts to control the spread of this important human disease.

Pneumonia Halted by Granulocyte Transfusions

In addition to conducting research on the causes and prevention of infectious diseases, NIAID scientists and grantees focus also on the treatment of these illnesses. Of particular concern are infections which, though comparatively rare in a normal person, are very serious in a patient with an underlying immune defect.

Such is the pneumonia caused by a type of bacteria known as pseudomonas. These organisms are relatively resistant to a number of antibiotics and cause often-fatal disease in patients with leukemia or other malignancies, aplastic anemia, or severe burns. Transplant patients who receive immunosuppressive drugs in order to give the grafted organs a chance to "take" are also at increased risk of pseudomonas pneumonia.

For a number of years, physicians have considered the possibility of using transfusions of granulocytes—certain white blood cells—to help restore the ability of these patients to fight a pseudomonas or other bacterial infection. Some recent clinical studies have suggested that repeated transfusions of normal granulocytes will, indeed, increase survival of immunodeficient patients with infections. However, no definitive controlled trials in man have been undertaken.

In order to determine if granulocyte transfusions have a clearly beneficial role, Dr. David Dale and his associates at NIAID last year developed an animal model system in which they were able to study the effectiveness of this procedure in an experimental fashion. Beagle dogs were given a high dose of radiation to knock out the majority of their white cells and then were infected with *Pseudomonas aeruginosa*, an organism causing pneumonia in up to 25 per cent of high-risk patients.

Twenty-four hours after infection, the test animals were randomly assigned to receive either an antibiotic, gentamicin, alone or in combination with transfusions of granulocytes from normal dogs of a different breed. Transfusions were continued for at least 7 days.

Results of this randomized controlled trial in an experimental animal indicated that granulocyte transfusions significantly prolonged survival, promoted clearance of the organisms from the lungs, and prevented or delayed the development of endotoxemia—a condition of shock caused by the release of harmful substances from the bacterial cells.

Although the NIAID scientists believe that their results strongly suggest that granulocyte transfusions may generally be useful in treating infections in immunologically compromised patients, they pointed out several considerations that should be taken into account. Among the most important, perhaps, was their observation that, though they found that granulocytes from genetically dissimilar donors could help the recipient fight an acute infection, there was some indication that granulocytes used in long-term transfusions should be matched for tissue compatibility. The scientists also believe that antibiotic other than gentamicin should be tried in concert with granulocyte therapy. Studies to compare such treatment regimens in the animal model are in progress.

Dr. Dale's clear demonstration of an effective role of granulocyte transfusion therapy in an animal model should prompt further clinical studies to define the specific circumstances in which granulocyte transfusions are of most benefit to man.

Drug Combination Effective Against Fungi

Systemic, or deep-seated, fungal infections involving the internal organs are also more serious in the immunodeficient or debilitated patient than in normal persons. Although most fungal diseases, such as histoplasmosis, cryptococcosis, and candidiasis, can be treated with amphotericin B or similar chemical compounds, these drugs are highly toxic and cannot always be given in fully effective doses.

Over the past few years, NIAID grantees at the Washington University School of Medicine, St. Louis, have developed a promising new method for treating serious fungal infections,

using a combination of drugs. Working with tissue cultures of a variety of pathogenic fungi, the scientists found that amphotericin B could be used to increase the anti-fungal effects of several antibiotics. The synergistic effect was particularly noticeable with rifampin, which inhibits synthesis of genetic material (RNA) and thus interferes with reproduction of the organism.

In studying the reason for this synergism, Dr. Gerald Medoff and his associates found that amphotericin B "made holes" in fungi cell membranes which enabled the rifampin to penetrate and interfere with RNA synthesis. Subsequently, the scientists have been testing the effectiveness, in the laboratory, of rifampin derivatives against various fungi, used singly or in combination with amphotericin B. In each case, the antifungal effect of the derivatives has been enhanced by use in combination with small doses of amphotericin B.

Unrelated Genes Can Be Transplanted

Research of far-reaching importance, but with a potential for both good and bad, was reported during the year by NIAID grantees at Stanford University in collaboration with other NIH-grantees at the University of California, San Francisco.

In November 1973 these scientists announced that they had found that genes from *related* bacteria could be combined in the test tube into a new molecule, then put in a bacterium to express themselves. In April, 1974, Dr. Stanley Cohen and Annie C. Y. Chang of Stanford reported that they could perform the same trick with *unrelated bacteria;* and, in May, the Stanford and University of California scientists brought a troubling scientific and ethical problem to a head by reporting their startling success in combining genes from a toad with those of a common bacterium.

Subsequently, in a rare departure from the ordinary course of scientific exploration, the investigators involved in these and similar studies of gene transplantation called on all scientists to join them in abandoning, or at least postponing, certain studies of DNA recombinants until efforts have been made to evaluate their potential hazards.

Although "gene transplants" have long seized the immagination of science fiction writers and intrigued scientists themselves, success in this line of research with bacterial genes began with the recent discovery of certain enzymes capable of cutting apart DNA in a way that produces fragments having adhesive ends. These sticky ends are then used to join DNA fragments from two sources.

The bacterial units of DNA used in the California experiments are known as plasmids—independent genetic elements within the bacterial cell. After the DNA of plasmids has been joined with genes from unrelated bacteria or from animal cells, the newly constructed molecules are inserted into bacteria such as *E. coli* —common inhabitants of the human intestinal tract. This insertion transforms the bacteria into essentially a new species, with genetic characteristics of both parent molecules. This new species can reproduce itself almost indefinitely, resulting in many billions of identical cells.

Scientists engaged in this research believe that the techniques they have developed might ultimately be useful in producing bacteria capable of synthesizing a wide variety of valuable biological substances, such as antibiotics or insulin. For agricultural use, bacteria capable of synthesizing chlorophyll or of substituting for fertilizer might be constructed.

On the other hand, it is possible that some types of gene transplants accidentally could make some common bacterial strains resistant to drugs or capable of producing substances toxic for man. In combining animal and bacterial genes, the possibility also arises that the animal genes might carry instructions for making tumor virus which, when attached to easily disseminated bacteria, might increase the risk of cancer.

In response to the scientists' request for a moratorium on this research, the National Institutes of Health has agreed to establish an advisory committee to evaluate biohazards involved and develop guidelines to be followed by investigators working with recombinant DNA molecules.

The National Institute of Arthritis, Metabolism and Digestive Diseases

Screening for Sickle Cell Anemia

Most states and many foreign countries are using dried blood specimens on filter paper to test newborn infants for the hereditary metabolic disease phenylketonuria, or PKU. This procedure now has been adapted by a National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) grantee, Dr. Michael D. Garrick of the State University of New York, Buffalo, to permit detection of sickle cell anemia and other hereditary hemo-

globin abnormalities. After extracting the hemoglobin from the dried blood specimens on filter paper, sophisticated chemical procedures are employed to distinguish the hemoglobin variants characteristic of sickle cell anemia and other hereditary disorders marked by abnormal hemoglobins.

The procedures are simple, convenient and inexpensive—the material cost of screening is reported as only three cents per specimen—and they permit screening of 250 to 500 specimens per day.

Fracture Healing Via Electric Current

In 1971 a NIAMDD grantee at the University of Pennsylvania, Dr. Carl T. Brighton, reported what was believed to be the first instance where healing of a previously refractory bone fracture was stimulated by a locally administered, mild electric current. Dr. Brighton now has detailed his experiences with more than 40 such cases in which patients have found their fractures healed in response to electrical stimulation by electrodes implanted within the bone near the fracture site.

Several other Institute grantees, notably Dr. Robert O. Becker of the State University of New York, Syracuse, and Dr. Morris H. Shamos of New York University, recently have reported similar successes. The technique offers hope for halving the healing time of some refractory fractures and may represent an alternative, in certain cases, for bone grafting.

Azaribine Therapy for Psoriasis

More than four million Americans are believed to be victims of psoriasis, a chronic and disfiguring skin disorder that is a source of mental anguish to affected individuals. At the University of Texas, Dallas, NIAMDD grantee Dr. James H. Herndon, Jr. has shown that azaribine, an anticancer drug, may be of value in treating severe generalized psoriasis that does not respond to other modes of treatment.

Thus far, azaribine has been used to treat experimentally more than 600 psoriatic patients in the United States and Czechoslovakia, where the drug was developed. Results have been good to excellent in three-fourths of the patients, and side effects of azaribine have been generally mild and easily controllable.

The duration of improvement following withdrawal of azarizine was highly variable, ranging from a few days to several months, but there was no accelerated rebound of disease activity as often occurs after withdrawal of potent steroid drugs.

Azaribine therapy for intractable psoriasis was introduced initially by a grantee of the National Institute of General Medical Sciences, Dr. Paul Calabresi of Brown University. His work has suggested that the vascular occlusive disease associated with psoriasis may also be ameliorated by azaribine thearpy.

Dr. Elizabeth F. Neufeld, NIAMDD, recently reported progress against the Hurler Syndrome and other inborn errors of mucopolysaccharide metabolism, on the basis of studies of cultured cells.

Pancreatic Cell Transplants in Diabetes

In 1972 it was reported by NIAMDD grantees Drs. Walter F. Ballinger and Paul M. Lacy of Washington University, St. Louis, that transplantation of insulin-producing beta cells from the pancreases of normal rats to diabetic animals results in significant reduction of blood and urine levels and restoration of weight gain in the lost rats. Although the disease was not cured, it was certainly modified and ameliorated. Inactivation of the transplanted beta cells was observed eventually, however.

Another Institute grantee, Dr. Leonard Lazarow of the University of Minnesota, now has developed a new approach to the problem of transplanting insulin-producing cells with a greater potential for their survival in the recipient. His work suggests that cells derived from the immature pancreases of newly-born animals can be transplanted to a diabetic animal more successfully than those from older animals. In the diabetic rat, for example, a single transplant of such cells was effective in controlling all symptoms of diabetes for more than one year.

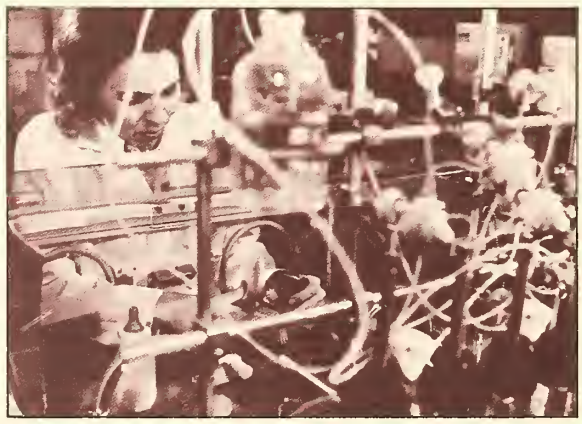
If this approach progresses to the point where effective long-term control of the symptoms of human diabetes can be maintained by transplanted beta cells, it will be important to determine whether such functioning transplants can prevent or delay onset of the complications of diabetes, primarily those affecting the blood vessels, kidneys, and eyes. If such complications are prevented, beta cell transplants might then provide a significant advance in diabetes treatment. Although such transplantation has indicated effectiveness in animals of the same genetic strain, consideration of research in human beings is regarded as extremely premature because of serious problems of tissue compatibility.

Removal of Bilirubin from Blood

Metabolic waste products and toxins are eliminated from the body predominantly by the kidney and liver. While artificial kidney therapy is an effective method of removing low molecular weight substances from the blood when kidney function is inadequate, there is no similar procedure for removing protein-bound substances, such as bilirubin, when liver function is impaired.

NIAMDD's Dr. Paul H. Plotz and associates, using bilirubin as a prototype for protein-bound substances, now have demonstrated a reduction of bilirubin levels in jaundiced rats greater than 70 percent in one hour, employing their affinity chromatography technique, which utilizes agarose beads coupled with human serum albumin. This procedure produced no observable ill effects and only minimal changes in formed blood elements, electrolytes and proteins.

The technique may prove useful for removing protein-bound substances from the blood of



patients with liver failure, in cases of intoxication with protein-bound drugs, or in specific metabolic defects.

Medical Control of Severe Internal Bleeding

Colonic diverticular disease is the most common cause of massive, acute lower gastrointestinal hemorrhage. Such bleeding usually can be controlled with conservative medical therapy and continued blood replacement. This is fortunate, inasmuch as those affected are usually elderly persons with coexisting diseases which make them poor surgical risks.

NIAMDD grantee Dr. Stanley Baum of Massachusetts General Hospital, Boston, has treated 15 patients with severe, unremitting diverticular hemorrhage who were considered medical therapy failures and thus candidates for surgery. Seven of the 15 patients had no recurrence of bleeding for six to 30 months following infusion of vasoconstricting drugs into the mesentery arteries which carry blood to the intestines. It is believed that Dr. Baum's novel technic may prove to be a valuable alternative to emergency surgical procedures.

New Blood Access Device

The arteriovenous (A-V) blood access device or "shunt" is a cannula that is perma-

nently connected to an artery and a vein in the arm or leg of a patient with kidney failure. It permits "hooking up" the patient to an artificial kidney machine repeatedly, without having to puncture an artery and a vein just prior to each treatment session. This technique was developed in 1960 by a NIAMDD grantee at the University of Washington.

Scientists at the University of Minnesota have devised an A-V shunt which, because of its shorter length and decreased resistance, allows three-times-faster blood flow than the standard equipment used in artificial kidney therapy. The device has been implanted in 32 patients who previously were unable to maintain functional standard shunts, and results have been so encouraging that the new device has been adopted as standard at the University of Minnesota Hospitals.

Dr. T. J. Buselmeier and NIAMDD grantee Dr. John S. Najarian, who developed the new shunt, believe that firm anchoring of the device prevents its tips from injuring the delicate inner lining (intima) of the blood vessels in which they are inserted and fixed. Faster blood flow is the principal factor that prevents blood sludging and, consequently, clotting. This factor, together with a decreased incidence of infection, provides for prolonged shunt survival in patients unable to maintain the standard shunt.

Unique Collagen Structure in Osteoarthritis

Osteoarthritis is characterized by destruction of cartilage in joints exposed to maximum mechanical stress, primarily in the weight-bearing joints. Recent research has shown that the connective tissue protein, collagen, present in normal joint cartilage, has a unique structure that apparently is essential to the mechanical strength and integrity needed for proper function of joints.

At the University of Southern California an Institute grantee, Dr. Marcel Nimni, has shown that osteoarthritic cartilage produces a form of collagen that is structurally different from that of normal joint cartilage.

The investigator suggested that the form produced in osteoarthritic cartilage weakens the collagen molecule and hence leads to premature joint destruction.

It appears that the different features of osteoarthritic collagen may account for some of the functional defects of cartilage in patients with osteoarthritis.

Vitamin A and Zinc

Several earlier studies have provided evidence to suggest that zinc may be essential to normal vitamin A metabolism. A NIAMDD scientist, Ernest G. McDaniel, and an Institute grantee, Dr. J. Cecil Smith, Jr., of George Washington University, now have shown in animal studies that zinc is necessary for normal

mobilization of vitamin A from the liver and for maintenance of normal plasma levels of vitamin A.

Moreover, the data obtained suggest that patients with depressed plasma levels of vitamin A, who are unresponsive to vitamin A therapy, may respond to zinc supplementation. When rats were fed vitamin A without zinc, the vitamin accumulated in the liver; but when zinc was added to the diet, liver vitamin A concentrations decreased significantly and plasma vitamin A levels increased markedly.

The National Institute of Child Health and Human Development (NICHD) holds a unique position within the National Institutes of Health. Research and training do not focus on a particular disease or biologic system. Rather, the NICHD is committed to the study of life as a continuum. It is by this life span approach that NICHD attacks some of the country's most important problems in child health, population studies, and adulthood*.

Benign Bacteria Protect Against Meningitis

Recent research by NICHD intramural scientists may be effective in lowering the incidence of acquired mental retardation, and provide a basis for revising current theories about how we acquire natural immunity.

Scientists led by Dr. John B. Robbins believe their recent findings strongly suggest that immunity against meningitis and other critical diseases may be safely stimulated by feeding newborns with a single dose of live, harmless bacteria.

Such immunity would be of great importance because the bacteria being studied—*Hemophilus influenzae* type b and groups of meningococci and pneumococci—can all cause meningitis, inflammation of the covering of the brain. *H. influenzae* type b, for example, causes about 12,000 to 15,000 cases of meningitis in infants and young children annually. Between 30 to 50 percent of the victims who recover after treatment with antibiotics suffer permanent disability, including mental retardation, making this disease the leading cause of acquired mental retardation in the United States.

Recently the scientists found that they could cause antibody formation against *H. influenzae* type b in animals by feeding them a harmless strain of *Escherichia coli*, bacteria normally growing in the human and animal intestine. The antibody response was great enough to protect against a deadly challenge from the *H. influenzae* type b organism.

Protection was due to a so-called cross-reaction response, which was possible because the capsular—or outer coating—antigens of *E. coli* and the *H. influenzae* type b are so similar biochemically that they evoke nearly identical antibody formation. The capsular antigens, when absorbed from the intestinal tract, act as signals to the body that antibodies should be produced to fight off a foreign organism.

Stimulated by the success with *E. coli* in conferring cross-immunity to *H. influenzae* type b, the scientists began a search for common, harmless organisms which would cross-react with other disease-producing bacteria, that is, Groups A and C *Neiserria meningitidis* and Types I and II *Diplococcus pneumoniae*. They have now found several harmless bacteria, the antigens of which cross-react with one or the other of these two bacterial types. Again, cross-reaction is due to similarity in biochemical structure. Growing the benign bacteria in the intestine of the animals produced potent antibodies to the disease-causing bacteria.

This finding suggests that some of the common, harmless bacteria of the intestine may be the source of natural immunity against many of the disease-producing bacteria developed by most persons before they reach late childhood. This concept differs from the traditional concept, which holds that natural immunity is evoked by a challenge from the disease-producing bacteria that is so mild as to cause no symptoms. The new concept could also explain why most adults and children over six years of age have serum antibodies to *H. influenzae* type b, although the organism is rarely found in nose, pharynx, or rectal cultures.

The scientists believe that it might eventually be possible to stimulate the growth of a colony of such harmless bacteria in the small intestine of very young infants (who have not developed their own natural immunity) in order to protect them from a host of bacterial diseases.

* Although a new, separate National Institute on Aging was authorized in legislation signed May 31, 1974, the NICHD will maintain its present interest in the study of aging as a developmental phenomenon.

They believe that a single feeding of the harmless bacteria would accelerate the development of adult immunity, usually not attained until about six years of age. Therefore, a child would be protected over the span of years between the time when antibodies acquired prenatally from his mother's circulation have ceased to guard him and the time he would otherwise fortuitously acquire natural immunity.

Vaccine Trial Against Meningitis

As mentioned earlier, although antibiotics do help children with meningitis caused by *H. influenzae* type b, 30 to 50 percent are still left with neurologic problems. Therefore, this same research group under Dr. Robbins has begun a trial vaccine program in Mecklenberg County, North Carolina, to prevent meningitis, and other disorders caused by *H. influenzae* type b, including inflammation of the epiglottis, pneumonia, a certain type of arthritis in children, and infection of bone. Preliminary results show that children protected in this manner have maintained normal health and have long-lived protection against the bacterium.

Epidemiologic studies by the scientists indicate why this vaccine program is especially important—they have shown that influenzal meningitis increased in Mecklenberg County from 9 cases in 1966 to 27 cases in 1971. This means that, unprotected, 1 of every 400 children in the county will have *H. influenzae* type b meningitis before the age of 5 years. If this rate applies to the entire country, then a similar vaccine program has the potential of saving over 8,000 children per year from attack and the after-effects of meningitis.

Although the incidence of influenzal meningitis has increased in recent years, the age distribution apparently has not changed. The disease most frequently affects infants and young children; its occurrence in newborns and adults is exceptional. There is a gap in the protective system between ages 3 months to 3 years—after antibodies from the mother have ceased to provide protection and before natural immunity has been acquired.

For this reason, all infants appearing at the offices of private pediatricians, general practitioners, county health department stations, or the out-patient clinic at the Charlotte (N.C.) Memorial Hospital for routine immunization are also being vaccinated against either *H. influenzae* type b or *N. meningitidis* Group C. The vaccine given against *N. meningitidis*—a bacterium causing another type of meningitis less common in the area than the influenzal type—is considered the control vaccine. Because protection is offered for all babies in a blind, randomized fashion, all infants benefit from the study.

Vaccine given the infants is not derived from the *H. influenzae* type b bacterium itself, but from a fragment of the capsule covering the bacterium. This fragment alone stimulates an antibody response sufficient to protect against attack from the bacterium.

Based on an expected 80 percent participation in the county scientists believe they will soon be able to prove statistically the benefit of such a vaccine program for use on a broader scale.

The NICHD scientists believe that eventually the two means of conferring immunity described above—feeding newborns with harmless bacteria, coupled with later vaccination with the capsular fragment—might provide optimal protection from *H. influenzae* type b, because there are indications that the latter type of immunization may be enhanced by the former technique.

Kittens Used to Study Infant Deaths

The institute also supports research to learn the causes and means of preventing another important contributor to infant mortality—the suddent infant death syndrome. A group

of scientists supported by an NICHD contract at the University of California, Los Angeles (UCLA), have found that studies in kittens are leading to more complete understanding of the complex syndrome that annually kills in the country 8,000 to 10,000 babies, usually between the ages of one month and one year.

The sudden infant death syndrome, sometimes called crib death, strikes without warning. In the majority of cases the baby is apparently in good health and feeds without difficulty. Although there may be evidence of a slight cold or stuffy nose, there is no history of serious upper respiratory infection. The infant is placed in his or her crib for a nap or for the night and several hours later is found dead. Although autopsy may show characteristic changes in certain tissues, none of them can be cited as a cause of death.

Why these babies die is as yet unknown. Many reasons have been offered to explain the deaths, including neurophysiologic, immunologic, cardiorespiratory, and infectious causes, but none has explained it fully. All factors believed to be associated with the syndrome appear to be intertwined.

The fact that the syndrome is nearly always associated with sleep has provided a research lead for several NICHD-supported groups including the UCLA scientists led by Dr. Dennis McGinty. Their studies in kittens indicate that the syndrome may be related to an interference with normal breathing patterns during sleep caused by a previous lack of sleep.

By continuously monitoring respiration, blood pressure, brain wave patterns, and tension in certain muscles of the kittens, the scientists note that the animals experience temporary halts in breathing (apnea) during slow-wave sleep and during rapid eye movement (REM) sleep—a particular period of "active" sleep that normally occurs several times during a sleep cycle, as shown in recordings of brain waves. Usually the kittens are able to rouse themselves from these apnea episodes and begin breathing normally again. Other studies show, however, that a substantial number of deaths occur in kittens between the ages of 2 weeks and 4 months, possibly having some relationship to these phenomena.

The monitoring system indicates that when kittens are deprived of sleep, the non-breathing episodes are more frequent. Moreover,

Dr. Louis Gluck and Mrs. Maria Kulovich, University of California at San Diego, extracting pulmonary lipids in studies of pulmonary maturation.

arousals from the episodes occur less often following sleep deprivation and during REM sleep, indicating that arousal mechanisms are depressed. Thus it is postulated that a prolonged apnea episode, occurring when arousal mechanisms are suppressed, could result in sudden death without pathologic findings.

In addition to the temporary breathing halts, the kittens are also prone to airway obstruction during REM sleep. Therefore, it is also possible that an airway obstruction in the hypopharynx or larynx occurring at a time when arousal mechanisms are depressed could cause irreversible respiratory failure.

The UCLA scientists are studying newborn kittens in connection with the sudden infant death syndrome because they seem to mimic, in many ways, situations occurring in human infants. For example, all infants are known to demonstrate breathing irregularities during their normal sleep and some infants have prolonged halts in breathing. These usually occur, as is the case in kittens, during transition into REM sleep. The newborn kitten is also ideal for such studies because, as the human infant, it is relatively immature at birth. Certain functions in kittens and infants develop significantly after birth, an important factor if immaturity of the central nervous system results in failure of the arousal mechanisms.

Feeding Sick Infants Intravenously

A group of NICHD-supported scientists at Columbia University College of Physicians and Surgeons is largely responsible for developing a method to feed sick infants until they are able to feed normally by mouth. This method is life-saving to infants with inborn malforma-

tions of the gastrointestinal tract and infants with severe diarrhea due to infectious diseases. In fact, feeding which by-passes the alimentary tract has increased the survival rates of these seriously ill infants from 30 to 80 percent.

Data gathered by these investigators, led by Dr. R. W. Winters, may eventually allow this feeding method to be applied to a much larger group of babies, that is, low birth weight infants, who often cannot obtain adequate nutrition in the first several weeks of life with usual feeding arrangements.

All essential nutrients—proteins, fats, carbohydrates, vitamins, and minerals—are delivered at a constant rate, via a catheter placed in a vein. The babies are closely monitored clinically and chemically. Daily adjustments in the nutrient solution are made.

Infectious complications have been as low as 5 percent and the infants show satisfactory weight gain, linear growth, skeletal maturation, and positive nitrogen balance—an indication of tissue growth. The weight gain is not due to water retention, but to an increase in lean body mass and fat deposition.

Nutrient requirements for babies fed by a means that by-passes the alimentary canal differ from requirements of normal infants. For example, the liver is no longer the "justifier" of absorbed nutrients, and microorganisms of the stomach no longer play a role in digestion and absorption. Because of this, totally new concepts of nutrient metabolism are necessary.

The Columbia group has found an optimal level of protein intake which allows the babies to grow well and maintain positive nitrogen balance, but which avoids problems of azotemia, that is, the retention of nitrogenous urinary waste products in the blood. In addition, they have defined optimal levels of calcium and phosphorus, important factors in the acid/base balance.

Some of their data will serve as a guide for the commercial preparation of "whole-diet" nutrient mixtures to be used for intravenous feeding of both infants and adults. For example, the scientists have found that if the mixtures contain a large load of potential acid, that is, materials which are converted into acid by the body, the babies will develop severe hyperchloremic metabolic acidosis. This condition can result in respiratory problems and renal failure in early infancy.

The group also solved another common problem of intravenously fed infants. Build-up of ammonia in the blood, known as hyperammonemia, can be prevented by supplementing the infused mixture with low levels of arginine, a by-product of protein metabolism.

Success in saving babies' lives and data gained about nutritional requirements of sick infants have led the investigators, with further support from NICHD and input from others working in the field, to develop recommended guidelines for intravenous feeding. These cover: identification of babies that can benefit from such treatment; specific aspects of catheter insertion; monitoring during feeding; and optimal levels of protein, fat, carbohydrate, vitamins, and minerals.

Effects of Adults on Young Children

The Institute is interested in the behavioral aspects as well as the biological aspects of child growth and development.

Research in the NICHD's laboratories indicates that personality and mental development at six months of age are independent of whether babies are cared for by their own mother full time, or by a combination of the mother and part-time caretaker. These findings, by Dr. Leon Yarrow and associates, are important in view of the fact that an increasing number of women with young children are joining the labor force. With many children from disadvantaged and middle-class families under care of a mother substitute part of the day, it is important to determine how surrogate care differs from mother care and how the differences might affect the children.

The NICHD investigators tested many of the factors that affect personality development in

Scientists study child's development of motivation to master her environment.

infants. On all 11 measures of stimulation examined, mothers scored higher than mother substitutes: They were more likely to play, to express positive feelings toward the infant, to place toys within the baby's reach, to provide a wide selection of playthings, and to interact with the baby in several ways.

But rather surprisingly, the infants cared for only by their mothers did not differ at six months of age in mental and personality development from babies with part-time substitute mothers. A possible explanation is that the working mothers, when they are able to spend time with their babies, may counteract the input from the surrogates by their additional attention to the child.

The importance of a father's relationships to his sons became apparent when the same method of assessment was applied in another study. Female infants, five to six months old, were not measurably affected by a lack of father interaction. Male babies who had more interaction with their fathers, however, at six months of age were more alert, responsive, and interested in their environment than those with little or no interaction.

The investigators speculate that the father may complement stimulation by the mother and introduce novelty into the infant's daily routine. Why only male infants seemed affected by absence of the father is puzzling. The study did not precisely explain the nature of the father's effect.

Behavior Modification for Delinquent Youths

The techniques of behavior modification have been widely used and proven successful in enhancing social adaptation of severely and mildly retarded and poorly adjusted children. Research initiated through the University of Kansas, Lawrence, jointly supported by the NICHD, the National Institute of Mental Health, and community funds, indicates that the techniques also can be applied successfully to treat juvenile delinquents, some of whom may also be mildly retarded. Data of the first several years of the project have just become available.

The Achievement Place Model, which was begun six years ago and has since been duplicated in 17 Kansas communities, as well as in other states, allows juveniles with behavior problems to be taken out of reformatories and institutions for the retarded and to be placed back in contact with the normal community. Presumably, community-based settings will allow them to take advantage of resources available for developing academic, social, vocational, and self-care skills.

Youths admitted to the Achievement Place homes have not been involved in serious law violations. They are, rather, youths who have been suspended from school, who have been in trouble in the community, or who are thought by their parents to be uncontrollable. Often they are functionally or educationally retarded.

The behavior modification technique used in the Kansas study is a system whereby the young people can earn daily or weekly points for good behavior such as cleaning their rooms or studying for an examination. These points can later be exchanged for privileges, for instance to allow attendance at a ball game or the movies.

The post-release behavior of the first 16 youths who were admitted to Achievement Place was compared with that of 15 similar youths who had been sent to a state boys' school and 13 who had been placed on probation. It was found that in two years after their release only 3 of the Achievement Place youths had again appeared before a court and returned to in-

stitutions, as compared with 8 of the boys' school group and 7 of those on probation.

Similarly, 14 of the Achievement Place youths still attended school three semesters after their release as compared with only 1 of the boys' school and 5 of the probation youths. For those who attended school, the Achievement Place youths received passing marks in over 90 percent of their classes while the boys' school and probation youths did so in less than 50 percent of their classes.

The Achievement Place Model also succeeded in terms of cost-benefit. The construction costs for Achievement Place type homes range from $6,000 to $8,000 per bed, as compared with $20,000 to $30,000 per bed for construction of state institutions for delinquent boys. Operations costs of Achievement Place homes run about $4,000 to $5,000 per youth per year compared with $8,000 to $10,000 for state institutions.

Project officers for the study were Drs. Montrose M. Wolf, Elery L. Phillips, and Dean L. Fixsen.

Development of Contraceptive Methods

Working with the support of an NICHD contract, the TECNA Corporation of Emoryville, California, has early data from trials of a new intra-uterine device (IUD) suggesting it may be an improvement over other IUDs.

This new IUD is a highly pliable, pouch-like, fluid-filled device that conforms to the shape of the uterine cavity. Its unique design is expected to eliminate undesirable side effects such as bleeding, and hazards such as perforations, which are problems in conventional "rigid" devices.

Preliminary data from the clinical trial conducted at University of California Medical Center, San Francisco, and parallel studies by private physicians indicate high effectiveness, low incidence of side effects, and good patient and physician acceptance, Dr. Sotiris Kitrilakis of TECHNA reported.

The pregnancy rate associated with the TECHNA device is 1.5 percent per 100 women-years (a woman-year being 12 menstrual cycles), expulsion rate 1.9 percent, and removal rate 14 percent. Published data on another commonly used intrauterine loop show that it has a pregnancy rate 3.5 times higher, expulsion rate 2.0 times higher, and removal rate for pain and bleeding 30 percent higher than the fluid-filled device.

No perforations occurred, probably due to the unique design of the new device, and incidence of pelvic inflammatory disease was 1.3 percent.

Women participating in the study were both those who have given birth (multiparous) previous to insertion of the device and those who have never given birth (nulliparous). In the nulliparous women—a more difficult group for IUD contraception—insertion was carried out with local anesthetics painlessly and with no recovery period. In the multiparous women insertion was performed with no anesthetic and little or no discomfort. Results with the nulliparous group may improve with use of a newly developed smaller model.

Other possibilities for fertility regulation have recently been suggested through basic research on how human reproduction functions on the molecular level. NICHD-supported scientists at the University of Michigan have elucidated a step in the reproductive process not heretofore recognized or known to be essential to ovulation, and subsequently to fertilization.

Ovulation—the release of the egg from a mature follicle of the ovary—is caused primarily by a large protein, luteinizing hormone (LH), secreted by the pituitary gland. Before ovulation can occur, the LH must bind to specific sites, called receptors, in the follicle. Dr. A. Rees Midgely, Jr., and his associates, have discovered that as the follicle grows and matures in preparation for ovulation, there is an increase in the number of receptors for LH. When LH has successfully bound to the receptors it sets up a chain of events that leads

to the release of the mature egg from the follicle; the egg is then moved to a position in the oviduct where it can be fertilized.

Dr. Midgely's work suggests that ovulation only occurs in those follicles which acquire threshold amounts of receptors for LH. If so, this step of the reproductive process would be a possible point of interception, and hence, contraception. A means of contraception at this phase of the reproductive process might be superior to other contraceptive means because it would closely mimic a normal process. During a woman's lifetime, relatively few eggs leave the ovary. Most remain within the ovary because the follicles which enclose them either fail to develop or develop partially and degenerate. Presumably, they do not develop sufficient receptors to bind LH. If this is true, drug-induced restriction or inhibition of LH receptors may lead to contraception by a mechanism that resembles what normally occurs, that is, degeneration of the follicle.

Aging Studies in Immune System

The immune system, made up of specialized cells, organs, and body chemicals, serves to protect individuals from invading organisms from outside the body, such as bacteria and viruses, or from deviant antigenic challenges from within the body, such as cancer cells.

The health of an individual depends upon an active immune system. This system is known to decline gradually with advancing age, thus leaving elderly persons more susceptible to infectious disease and to disorders such as cancer and other autoimmune diseases, which increase in incidence with age.

Scientists at the NICHD Gerontology Research Center, led by Dr. Takashi Makinodan, are investigating aspects of immunity through studies with aging populations of mice. They are beginning to pinpoint where in the complex immune system this "wearing-out" process might begin.

The decline in immunologic activities—immunosenescence—can be due to changes in the cells of the immune system, changes in their environment, or a combination of the two. In order to investigate this, the scientists cultured lymphoid cells of old and young mice donors in young and old recipient mice. If the immunologic decline is due primarily to changes in the cells themselves, then old cells would be expected to perform poorly in young recipients, and young cells perform vigorously in old recipients. If the decline is due primarily to changes in the cellular environment, then old cells should perform vigorously in young recipients and young cells poorly in old recipients.

The scientists found both factors are responsible, but that the cells account for 90 percent of the decline and the cellular environment, or humoral aspects, only 10 percent.

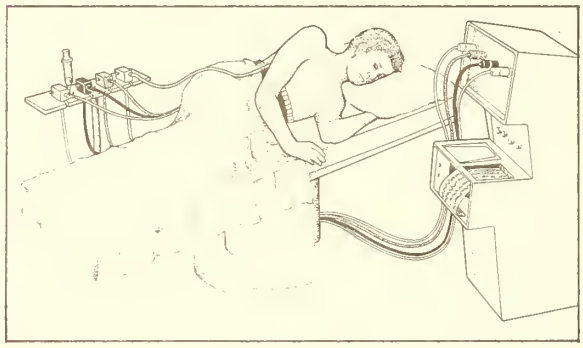
Other studies of this group have shown that the activity of bone marrow stem cells declines with age. That is, the stem cells lose their ability to differentiate into T cells (those formed in the thymus) and B cells (those formed in the bone marrow). These T and B cells are lymphocytes necessary for an effective immune response. With aging, these T and B cells lose their ability to proliferate efficiently in response to antigen stimulation and give rise to functional progeny. Another type of cell, the A cell or accessory cell, appears to be unaffected by aging. These A cells in some way cooperate with B and T cells in response to a challenge by an antigen.

In other recent work, the investigators studied possible underlying causes for the inability of the T and B cells to produce effective, potent cells in numbers great enough to respond to a challenge. They found that the inability may be due to the presence of a certain type of repressor cell, known to be more common in old than in young mice. The Center scientists are attempting to correct and explain the defect in this part of the immune system by removing the repressor cells.

Conditioning to Treat Fecal Incontinence

An NICHD study at the Gerontology Research Center, Baltimore, indicates that patients with chronic severe fecal incontinence manifested by daily soiling can be trained to become continent. This is so even when the incontinence can be attributed to organic lesions—for example, following rectal surgery.

This study is from an ongoing program led by Dr. Bernard T. Engel. Other studies in this program already have shown that human patients with abnormal heart rhythms can be taught to control these rhythms, and that monkeys can be trained to control their heart rates.



In their study of incontinence, Dr. Engel and his associates first noted that all of the patients lacked a normal reflex called the recto-sphincteric reflex. In this reflex, stretching of the rectum—which would occur when a stool enters the rectum—leads to the relaxation of the internal sphincter, a muscle ring just above the anal passage. The reflex also causes contraction of the external sphincter, a muscle ring at the end of the anal passage. Relaxation of the internal sphincter permits the stool to pass into the rectum, and contraction of the external sphincter prevents the stool from being expelled. When they entered the study, none of the patients had a measurable external sphincteric response.

Following training, however, all of the incontinent patients learned to control their responses within four training sessions. Furthermore, follow-up studies as much as five years later showed that these patients remained continent.

Training consists of showing a patient tracings of his sphincteric responses at the same time that his rectum is being artificially distended by a balloon. Patients are given encouragement whenever a normal response is made, but verbal reinforcement is gradually decreased as it becomes clear that the patients are able to control their sphincters.

These findings are important because they indicate means of treating a problem for which there is no consistently effective alternative therapy. In addition, the success suggests that training using the methods developed by these investigators, might be extended to the treatment of functional disorders of other systems characterized by responses heretofore believed to be entirely involuntary.

Factors Predisposing to Peptic Ulcer

In a society in which most infectious diseases are controlled, chronic diseases such as stroke, cancer, coronary heart disease, and others, have become the leading causes of illness and death, particularly among older persons.

An NICHD-supported investigation, through the University of California at Berkeley, has identified certain characteristics of college age men which predispose them to increased incidence of specific diseases in later life.

The most recent data gathered by Dr. Ralph S. Paffenberger and his associates indicate that habitual coffee drinking is a better predictor of later peptic ulcer than any of the other characteristics they studied, and milk drinking is common to those persons most likely to remain ulcer free. The apparent relation of coffee drinking to peptic ulcer suggests that breaking a coffee-drinking habit might forestall or prevent development of this serious disorder.

Other factors that appear related to peptic ulcer are: soft drink consumption, tobacco smoking, lower systolic (pumping) blood pressure, less participation in physical activity and varsity sports, and a history of indigestion and diarrhea. Surprisingly, neither tea nor alcohol consumption in college seems to influence occurrence of ulcer in later life. The study turned up no personality or psychological traits distinguishing the ulcer prone from other students.

Data on peptic ulcer and other diseases studied by these investigators are based on medical records of some 50,000 men who were in college between 1916 and 1940, plus questionnaires, case-studies, and death certificate studies 16 to 50 years later.

Other data gathered by this group identify characteristics which predispose college age men to an increased incidence of old-age-onset diabetes mellitus. These predisposing characteristics were higher systolic blood pressure, lower lung capacity, a parental history of diabetes mellitus, and greater weight-for-height. Greater weight-for-height in college seemed the best predictor of subsequent diabetes. Presumably, reduction of obesity through diet or exercise might forestall the onset of this disease.

In another part of the study, predisposing characteristics of coronary heart disease were identified. These were cigarette smoking, high resting and pumping blood pressure, physical inactivity, greater weight-for-height, shortness in stature, sense of exhaustion, and early death of parents. Again it appears that if weight, smoking, and blood pressure could be reduced, incidence of coronary heart disease might decrease.

As the chief sponsor of dental research and the training of dental scientists in the United States, the National Institute of Dental Research is interested in studies ranging from the causes of diseases of the mouth to the development and application of better methods for treating and preventing them.

Today investigators in over 30 different branches of science are contributing an ever growing base of information on which the Institute is building. The oral diseases are so complex that solutions to the questions they raise require the cooperation of clinicians, epidemiologists, and laboratory workers. A biomaterials scientist, a microbiologist, an engineer, and an embryologist may all have to work together in order to solve a particular problem, for example.

The findings reviewed here illustrate the basic research conducted and supported by NIDR to improve the oral health of the Nation.

Caries Vaccine—Problems and Prospects

A relatively recent Institute approach to caries prevention is exploration of the possibility of a caries vaccine. The injection of killed bacteria in rats gave encouraging but variable protection against tooth decay when the animals were later inoculated with the same kind of bacterium. Dr. Jason M. Tanzer, now at the Veterans' Administration Hospital, Newington, Connecticut, collaborated with his former NIDR colleagues, Dr. George J. Hageage, Jr., and Dr. Rachel H. Larson, in a study of a vaccine containing *Streptococcus mutans*. This organism, derived from human caries lesions, readily destroys rat teeth when animals are infected with it and fed a sugar-rich diet. The bacterium attacks not only the smooth, vertical sides of the teeth but also crevices in their chewing surfaces.

The ability of *S. mutans* to convert the dextrose half of the sucrose sugar molecule into an insoluble, sticky form of dextran is believed to be very important in the formation of plaque, a bacterial film that collects on teeth and accumulates if not removed regularly. Bacteria found in root plaque differ from those most common in crown plaque, and it appears that certain types of plaque are also closely linked with periodontal disease, which causes deterioration of tissues supporting the teeth.

The caries vaccine acts in two ways. First, it stimulates formation of the usual antibodies in the blood stream that provide immunity against most bacterial infections in the body. Second, and more significantly, it increases the secretion of antibody in saliva, the only body fluid that bathes the bacteria on teeth. Immunoglobulin A (IgA), the important antibody in saliva, prevents infections without killing bacteria or reducing the total amount of plaque. Rather, as other NIDR-supported research by Dr. Ronald Gibbons at Forsyth

Teeth of rats fed a sugar-rich diet and infected by *S. mutans* derived from human carious lesions, are readily destroyed.

Dental Center, Boston, has shown, IgA can make certain bacteria clump together and thus interfere with their attachment to various tooth surfaces.

Dr. Tanzer's group is perplexed by the inability of their vaccine to immunize their rats consistently. Moreover, some of their animals developed sores where they were injected under the skin. Accordingly, other dental scientists are now using monkeys, which have a larger variety of organisms and react more like humans than rats do. Studies now in progress should show whether an inoculation for just one organism can appreciably and safely reduce decay in subhuman primates. Only then can vaccines be considered ready for human testing.

Saliva Protects Against Disease

Compared to blood or urine, saliva is a poorly understood body fluid. Its content varies genetically and with the state of health. Different sets of salivary glands produce chemically different secretions. IgA antibodies can prevent some bacteria from attaching to tooth surfaces, as previously noted, and specific salivary molecules also temper the ability of a large number of bacteria to attach to the surfaces of the epithelial cells that line the mouth and the rest of the alimentary tract. Moreover, the constant flow of saliva mechanically removes many bacteria.

Bacteria cannot cause disease unless they are able to settle, multiply, and accumulate by-products harmful to their host. The predilection of specific bacteria for certain places is well known. For example, diphtheria attacks the back of the throat, not the tongue. *Streptococcus salivarius* attaches by the hundreds to epithelial cells that line the cheek, but only a few of this species appear on epithelial cells covering the jawbones.

In searching for mechanisms of attachment, scientists at the Forsyth Dental Center turned to a class of molecules in which proteins are bound to complex sugars. Since some of these glycoproteins form the molecular bases of the familiar "blood group" antigens on cell walls and also serve as attachment sites for certain viruses and Mycoplasma organisms, Drs. Ray C. Williams and Ronald J. Gibbons reasoned that these surface antigens might also anchor bacteria.

Earlier, the scientists had showed that some of the "blood group" glycoproteins also occur in saliva and bind selectively to certain bacteria, making them clump together. Next, they demonstrated that, by occupying the attachment sites of certain bacteria, these glycoproteins could prevent the organisms from attaching to epithelial cells. The investigators found that saliva even detached bacteria that had already fastened to epithelial cells.

In other experiments, epithelial cells were scraped from the inner surface of cheeks, washed, and exposed to various species of streptococci. Both *S. sanguis* and *S. salivarius* clung to these epithelial cells so closely that further washing could not remove them. However, if the epithelial cells were first treated with antisera to the blood group protein, far fewer streptococci could attach. Antibody in the antisera had covered the binding sites which the glycoproteins normally exposed on the cell surfaces. In this way Drs. Williams and Gibbons proved that salivary glycoproteins can compete with those on cell walls as bacterial traps.

Dr. Gibbons also pointed out that, because surfaces of cells in different regions of the body have different collections of antigenic glycoproteins, it is understandable that specific bacteria colonize particular places. Since these glycoproteins are genetically determined, they may be responsible for the resistance or the susceptibility to certain diseases that appears to correlate with blood groups.

Bacterial Evolution and Disease

Of the many forms of bacteria in the mouth, some cause disease, some are harmless, and some may even be useful. It would be helpful to know why seemingly similar organisms differ so greatly in their effects. To show evolutionary relations among bacteria, small enzyme molecules that perform almost identical digestive functions in a number of different bacteria, and which operate under similar control systems, are being analyzed by an Institute scientist.

Dr. Jack London finds that the borderline between the harmless and harmful may be only the width of a few molecules. He hopes that studies of bacterial evolution will explain how disease-causing types develop from free-living relatives that apparently survived on simple inorganic substances for over two billion years.

Traditionally, the evolution of higher plants and animals has been traced by comparing living species with fossil remains for similarities and differences in form, but bacterial structural forms are few, and have not changed over millenia. One test, the Gram stain, does separate all bacteria into two different kindreds. Organisms with walls that accept the stain are in a separate category from those which reject it. Beyond this division, most bacteriologists have relied on growth habits, food substrates, and digestive waste products to assign an organism a place within a few large groupings by shape, such as threads, rods, or spheres.

Dr. London considers that most bacterial growth habits are no more relevant to their evolutionary distinctions than similarities of diet and metabolic products would be for distinguishing among pigs, baboons, and human beings.

A better evolutionary tool, Dr. London maintains, is an enzyme. Preferably, the molecule should be a small one that is essential to many kinds of bacteria for the digestion of a widely available type of food. When large chunks of such a molecule are exactly alike in different species, the similarity indicates that the enzyme occurred in the most primitive organism and has been conserved over the ages. Because the enzyme works so efficiently, new hereditary changes or mutations are likely to be harmful, and therefore are not

perpetuated. Scientists believe that the more alike such digestive molecules are in different species, the more closely the species are related. They also presume that a large molecule which controls a simple reaction could mutate and survive more easily than a small one which catalyzes an essential, more complicated reaction. It would be most surprising if a small enzyme that requires more than one binding site on the molecule could mutate and still function.

At present Dr. London has limited his research to Gram-positive bacteria which require an animal host. He noticed that only two species found in the mouth grow on malate, a salt of malic acid found in many fruits. One, *Lactobacillus casei,* is rod-shaped, and associated with tooth decay. The other organism, *Streptococcus faecalis,* is a sphere that generally lives harmlessly in the intestine; some strains can cause abscessed teeth and rheumatic heart disease, however.

A comparison of malic enzymes from ten strains of *S. faecalis* and 18 strains of *L. casei* showed that these molecules were partly alike in all strains because they cross-reacted immunologically. All of the *S. faecalis* malic enzymes also were similar in electric charge and molecular weight. However, the *L. casei* enzymes fell into two groups differing sharply in electric charge, some being more and others less electronegative than the *S. faecalis* enzymes.

These findings indicate to Dr. London, that, despite differences in shape between lactobacilli and streptococci, these particular organisms all shared a common ancestor. At a later date, the parent lactobacillus gave rise to two modern sub-species, which probably should now be separated.

In similar work, Dr. London has shown that the aldolase enzyme is another good molecular tool for grouping bacteria into family lines. It splits compound sugars and releases energy for all bacteria that make lactic acid as a digestive waste. A few of these species comprise half the inhabitants of the mouth. Dr. London can now show which aldolase-producing bacterial lines contain only harmless passengers, and which have developed some dangerous members.

Protein Deficiency Increases Rat Caries

Although experimental and field studies show that diet plays a very important role in tooth decay, relatively little research on the effects of proteins in caries has been conclusive.

Recently, however, Drs. Juan M. Navia and Lewis Menaker of the University of Alabama's Institute of Dental Research in Birmingham showed that offspring of rats kept on a low protein diet during pregnancy developed much more tooth decay after weaning than offspring of mothers fed a protein-rich

Two "healthy" rats of the same age. Smaller rat is stunted by protein-deficient diet.

diet. To measure the effects of protein on tooth development, the investigators hand-fed some of the nursing pups with a protein supplement, and others with a protein-free supplement equivalent in calories, minerals, and vitamins. After weaning, all the animals were fed a diet with protein adequate for good growth, but also with abundant sugar to promote tooth decay.

As expected, offspring of protein-deprived mothers weighed about half as much at birth as those of well-nourished mothers, and never achieved more than three-quarters of the normal adult weight. But more importantly, they confirmed Dr. Navia's suspicion by developing far more caries than their well-fed contemporaries.

Pups given extra protein during the nursing period while teeth were forming weighed only 10 percent less than the advantaged pups and had as much resistance to decay as the pups of well-fed dams. Pups which received plenty of non-protein calories grew better than unsupplemented pups but later suffered the same severe caries.

Dr. Navia believes that the tooth decay ex-

perience of his rats parallels that of people in many parts of the world whose poor oral health and stunted growth he attributes to diets containing too little protein and too much sugar.

Fluoride is Essential

The latest addition to the catalogue of nutritional essentials for life is the element fluorine.

Trace elements, which function in very minute amounts, are easily overlooked and very hard to test. This difficulty is illustrated by the fact that scientists working under a grant from NIDR had to observe mice over a period of more than four generations before they could establish that a fluoride-deficient diet reduces reproductive capacity. With small amounts of a fluoride salt in the diet, the rate of successful pregnancies in mice continued to be at least 96 percent. However, on a very low fluoride diet that was otherwise identical, the rate dropped to 48 percent after the fourth mating. Then, when the fluoride-deprived mothers were switched back to the fluoridated diet, their reproductive capacities returned to normal.

Dr. Wallace D. Armstrong and his associates at the University of Minnesota say that their study "demonstrates that fluoride satisfies the major criteria for an essential trace element: a deficiency state has been produced in mice on a diet low in fluorides; the deficiency is prevented and cured by addition of fluoride alone to the diet."

The scientists also found more severe anemia in the fluoride-deficient mice during pregnancy than in mice fed adequate fluoride. They suggest that perhaps the anemia contributed to the decline in reproduction.

Local Fluoride Benefits Exceed Systemic

Contrary to most current thinking, animal research at NIDR indicates that frequent exposure to low levels of fluoride in drinking water gives much more protection against tooth decay that the incorporation of large amounts of fluoride into enamel during or after tooth formation. The mechanism by which fluoride acts locally is unknown, but several scientists have suggested that it may interfere with the working of some bacterial enzymes.

It is well established that, during the formation of bones and teeth, fluoride salts which are absorbed systematically through the intestine improve the size and structure of the mineral crystals that form the bulk of bones and teeth and make that mineral less soluble in acids. The protection against tooth decay that fluoride provides has been attributed chiefly to these systemic effects on tooth enamel. Many scientists have therefore assumed that the reduction in tooth decay that followed an increase in the fluoride content of the outermost layer of enamel by local applications of strong fluoride solutions or gels was due to the improved resistance of enamel to bacterial acids.

Now Dr. Rachel H. Larson and her colleagues of NIDR, and James R. Mellberg of the Kendall Company, Barrington, Ill., report evidence that local effects of fluoride are far more beneficial than its incorporation into enamel, although both are helpful.

For a week Dr. Larson kept two groups of weanling rats of the same age and strain on a low-fluoride, starch diet that does not encourage decay. In this pre-test period one group drank water without fluoride, while the water of the other was strongly fluoridated. Analysis of the erupted first and second molar teeth showed six and one-half times more fluoride in the enamel of the second group after that week's exposure.

For the next 56 days of the test period, all the animals were put on a sugar-rich diet after being infected with a strain of *Streptococcus mutans* taken from a decayed human tooth. During this period the original groups were subdivided so that half of each received water without fluoride and the other half drank water with ten parts per million of fluoride. This amount of fluoride is excessive for human drinking water, but less than was formerly thought desirable for rat studies. At the end of the experiment, the rats' teeth were analyzed for fluoride, and the decayed areas were counted so that the four groups (advantaged, deprived, local-action, systemic-action) could be compared for fluoride effects.

As expected, the advantaged rats, that received fluoride in both the pre-test and test periods, developed the least decay.

The deprived rats, that received no fluoride at all, had more than twice as many decayed areas as the systemic-action group which drank fluoridated water only during the pre-

test week. The local-action rats, which had no fluoride during the pre-test week and only low levels in their water during the 56-day test period, surprised the investigators by developing still less decay than the systemic-action group. Moreover, tooth analysis at the end of the experiment showed that the teeth of the local-action group had acquired very little more fluoride than was found in the teeth of the deprived animals which consumed no fluoride during the test period.

Since such important factors as heredity, age, diet, and bacteria remained the same in this experiment, the results show clearly that daily exposure to low levels of fluoride in drinking water protects far more against caries than does fluoride incorporation into enamel, whether by local application or by systemic absorption.

Which Trace Substances Affect Caries?

Water fluoridation for caries prevention belongs in the category of natural protective agents. Today the search for a new agent against tooth decay may be quickened by improved analytical tools, but before any new substance can be pinpointed as protective, a scientist first must suspect its value.

The index of suspicion increased sharply a few years ago in the course of an NIDR-supported study of alternate ways of providing fluoride protection in certain low-fluoride areas in Colombia, South America, where there are no central water supplies.

Many small towns isolated among high mountain ranges in Colombia have very stable populations almost identical in genetic stock, size, socioeconomic conditions, numbers of children, and food habits. In these communities the average level of natural fluoride in springs and streams is not more than 0.1 parts per million. Preliminary surveys of the suitability of several towns for testing the benefits of adding fluoride to table salt disclosed that one town was strikingly different. In Heliconia, the rate of decay is surprisingly low—only 5.5 decayed, missing, and filled permanent teeth per child, as contrasted with a figure of 13.9 in children of the same age in Don Matias, where the rate of decay is comparable to that elsewhere in much of South America.

Puzzled by such a difference in otherwise highly similar towns, dental epidemiologists wondered whether one or more trace substances other than fluoride might account for better dental health in Heliconia. Conversely, might other compounds increase decay in Don Matias and elsewhere?

To seek answers to these questions, 21 elements were studied in soil and water samples from the two communities. Spectroscopic analyses of the soils showed local differences in the distribution of some trace metals, but dietary histories and studies of the sources of common foodstuffs support the hypothesis

Dieticians take dietary history of families in Colombian Villages.

that any effects of trace elements are more likely due to differences in drinking water than in food.

Thirteen elements were present in similar amounts in the waters of both towns. Titanium was slightly, but not significantly, higher in Don Matias. However, levels of copper, iron, and manganese were considerably higher in Don Matias. In contrast, the levels of calcium, magnesium, and vanadium were significantly higher in Heliconia.

Previous studies of correlations between the presence of trace elements and the amount of caries in a community have rarely excluded the effects of fluoride. But a few surveys have suggested that hard water may be associated with reduced tooth decay. Such waters contain more than average amounts of calcium, magnesium, and molybdenum. Also, there is very limited evidence that excess copper and manganese may promote decay.

Before any conclusion can be drawn that a particular substance is useful or detrimental in such a complex disease as caries, much more research is required to separate the effects of that compound from the actions of others. However, fifty years ago no one speculated that a mere one part per million of any substance in drinking water could reduce a worldwide disease. Yet fluoride at that concentration gives today's best protection against tooth decay. Perhaps other trace substances may also prove beneficial.

This preliminary investigation was made by Drs. Robert L. Glass of Forsyth Dental Center, Kenneth J. Rothman of Harvard University, F. Espinal, and Hernan Velez of the University of Antioquia, Medellin, Colombia, and N. J. Smith of Dow Chemical Company, Midland, Michigan.

Prostaglandins Influence Alevolar Bone Loss

A partial explanation of the loss of alveolar bone that occurs in severe periodontal disease has come from the University of California in San Francisco. Alveolar bone forms the ridges of the jaws and the sockets that support the teeth. Its disappearance in the later stages of periodontal diseases leaves no support for natural teeth or, in many cases, even for dentures.

Dr. Jo Max Goodson found ten times the normal level of certain prostaglandins in patients with severe periodontal disease. These biologically active substances, resembling hormones, were concentrated in the inflamed tissues that cover the ridges of alveolar bone and surround the teeth. Unlike hormones, which are synthesized in glands and usually affect target tissues elsewhere, prostaglandins are synthesized in many parts of the body where they act directly.

Dr. Goodson has shown that, in tissue cultures, levels of certain prostaglandins, equivalent to those he found in the inflamed tissues, cause rapid disappearance of bone. He reasoned that substances known to inhibit prostaglandin synthesis might help patients with peridontal disease.

The University of California has supported further studies by Dr. Goodson which indicate that daily doses of vitamin E, a known prostaglandin inhibitor, reduced inflammation in a small group of patients with peridontal disease. Although control of bone loss cannot be demonstrated in this human study, the vitamin does appear to reduce inflammation.

Why Fever Blisters Recur

The common fever blister, usually considered more of an annoyance than a danger, is caused by Type 1 of the herpes simplex virus. However, the virus can be dangerous when it infects the eyes, or the skin after burns, or when it attacks people taking immunosuppressive drugs. It may even be fatal to infants with a systemic herpes infection. Moreover, its close kin, the Type 2 herpes virus, causes a common veneral disease recently linked to cancer.

Both kinds of herpes infections differ from most others in that they tend to repeat even when high levels of antibody circulate in the blood. The mechanisms which enable herpes to recur are being studied by Dr. Abner L. Notkins and others at NIDR.

Viruses can grow and multiply only in cells. When they have matured, the cell wall breaks, releasing new viral particles to infect neighboring cells. In defense against foreign invaders, the body makes antibodies individually tailored to each enemy. By surrounding the viral units, these antibodies inactivate them so that they can no longer infect cells. Dr. Notkins has discovered that, despite being bound to antibodies, a very small percentage of herpes particles remain infectious.

Presumably, virus particles are protected while enclosed in the cells which they have infected. However, a few hours after infection, antigens appear on the walls of infected cells and betray the presence of the virus. These signal antigens call up a series of serum proteins, collectively named complement, to react with antibody. Together, complement and antibody then destroy the infected cells and their viral contents.

Yet some of the tiny viral units still manage to escape this second defense. Before the antigen develops on the outer wall, a few viral particles quickly pass through a "bridge" directly from cell to cell. Thus, they avoid detection and escape the executioner proteins, both antibody and complement.

The body has a third defense in certain white blood cells. When activated, these leukocytes migrate from blood vessels into tissues and there remove body refuse, such as dead cells, bacteria, antigen-coated cells, and virus-

antibody complexes. The virologists at NIDR reasoned that if a large number of activated leukocytes can kill infected cells, perhaps a smaller number would still be sufficiently toxic to control a virus infection, either by arresting the metabolic machinery of the cell enough to prevent the virus from using it to multiply, or by preventing the formation of bridges between the cells.

The investigators found in culture preparations that a limited number of activated rabbit

Using a laminar flow hood, a virologist conducts tissue culture studies with herpes simplex virus.

leukocytes would permit herpes-infected rabbit-kidney cells to survive with no evidence of viral spread, but three days after the leukocytes were removed, the virus multiplied again and spread. Therefore, the white cells alone, without antibody or complement, had temporarily checked but not cured the infection. Likewise, antibody and complement, in the absence of leukocytes, could not stop the infection. The infection was cured only when the scientists allowed white cells to inhibit the infected kidney cells briefly, then added antibody and complement to the culture in the short interval before bridges could reform. Later, when the investigators removed the white cells, the kidney cells continued to live and grow normally.

These experiments suggest that protection against herpes, and probably against other recurrent viruses, requires an ability to make antibody, a complete, active system of complement enzymes, and the capability to activate and attract leukocytes quickly to sites of infection. A deficiency in any one of these three immune responses could lead to recurrent infections.

Can a Virus Trigger Diabetes?

Virologists at NIDR are also studying the effects of viral infections on the secretory processes of salivary glands and of the pancreas, which makes similar amylase enzymes that convert starch into sugars. Diabetes is a pancreatic disease characterized by abnormal processing of carbohydrates. Some researchers hypothesize that certain forms of diabetes have a viral origin because patients have reportedly developed diabetes shortly after such infections.

In 1968 scientists at the University of Vermont discovered that encephalomyocarditis (EMC) virus can induce a diabetes-like disease in mice. This lead has been followed by Drs. D. Wark Boucher and Abner L. Notkins of NIDR, who now conclude that certain mice having this infection may prove to be good models for studying diabetes.

The virologists found that males in two of seven strains of mice are susceptible to EMC virus and that, following this infection, many of them develop metabolic symptoms of diabetes. The virus, they report, rarely causes problems in females of the two susceptible strains and produces no diabetic symptoms in infected males or females of the other five strains. Therefore, viral damage seems to depend on genetic factors determining sex and strain.

The ability to handle a load of sugar was impaired in nearly all of the males according to glucose tolerance tests. Many animals had sugar in their urine and displayed the excessive hunger and thirst that afflicts diabetics. In most of the affected mice, blood sugar reached high levels that dropped fairly soon, but 10 to 15 percent of them remained hyperglycemic for over six months, a long time in the life span of a mouse.

Insulin is made in beta cells in areas of the pancreas called islets of Langerhans. The diabetic mice had fewer islets than usual, and most of these were shrunken and distorted. Tissue sections treated with fluorescent dyes indicated that viral antigens were present in the beta cells. Therefore, the investigators conclude that the beta cell damage was a direct result of the EMC viral infection.

The National Institute of Environmental Health Sciences

Quail eggs exposed to microwave radiation to help determine its effect on the developing embryo.

The number and variety of toxic substances released into the environment as a result of modern technology increases year by year. Because of the nature of our society, we become more and more dependent upon technology for jobs, transportation, control of diseases, production of food, fiber and energy, and the preservation of foods and material. No segment of the population can escape some contact with potentially dangerous substances.

It is imperative, therefore, as recent history has often demonstrated, to identify potentially dangerous agents in the environment, or proposed for future release, and to understand the consequences of human exposure to them in terms of overt and covert diseases and physiological disorders. Knowledge is the first defense against further degeneration of environmental quality with its consequent toll on the quality of human health.

To develop such knowledge and understanding, the National Institute of Environmental Health Sciences (NIEHS) was created in 1966. NIEHS research is aimed at the solution of health-related environmental problems. Its goals are to develop understanding of the interrelationships between chemical and physical factors in the environment and human diseases, and to help translate the knowledge developed into planned action to diminish or relieve human problems of environmental origin.

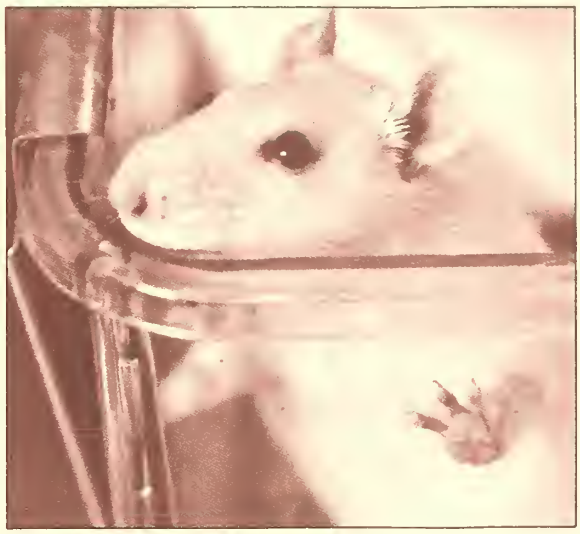
Halo Ethers

Studies by a team of scientists supported by an NIEHS Center grant at the New York University Medical School Institute of Environmental Medicine have resulted in identification of a significant hazard to human health that now can be controlled or removed from our environment.

Several years ago, the scientists, led by Dr. Norton Nelson, began studying bis chloromethyl ether, one of a family of organic chemicals commonly called halo ethers. These compounds are used in large quantities in the chemical industry for polymerization and stabilization of polymers. The NIEHS grantees suspected, on the basis of the chemical structure of the bis chloromethyl ether, that these compounds might be carcinogenic (capable of producing cancer).

Testing the compounds supported their suspicions—bis chloromethyl ether was indeed a potent carcinogen when applied to the skin of mice or when injected into mice or rats. Continuing studies showed that after inhalation of the compound in very low concentrations the mice developed lung tumors. This was particularly important because workers in the chemical industry probably were exposed to fumes of the compound.

These data were published in scientific journals and were confirmed in other laboratories. In addition, the NYU group, in concert with the



NIEHS and the National Cancer Institute (NCI), considered these findings of such significance that a conference, partially supported by NIEHS, was called to review the entire class of halo ethers and to answer questions as to the present and projected use of the compound and the levels and circumstances of human exposure.

Representatives of NIEHS, NCI, the National Institute of Occupational Safety and Health (NIOSH), and the industries involved in the manufacture and use of these compounds attended the conference. As a result of this information exchange, one manufacturer immediately stopped production of the halo ethers and other manufacturers took direct steps to minimize exposure of their workers to the compounds.

NIOSH undertook studies to evaluate the effects on humans and found presumptive evidence that lung cancers occur in workers exposed to bis chloromethyl ether. To define the problem in terms of quantitative dosage responses, NIOSH provided additional funds for further studies on humans. At the same time,

experiments were undertaken to develop safety criteria and standards on which to base realistic control measures, and for use by the Department of Labor in surveillance and control actions. Concomitantly, the NCI provided funds to support research on determining the relationship between molecular structure and carcinogenicity of the halo ethers as a class.

NIEHS continued, and is continuing, to provide basic support to the NYU group to determine the mechanism by which the halo ethers induce cancer. This information is necessary to develop a rationale for more effective prediction of the carcinogenicity of chemicals and the levels and circumstances of exposure that can be expected to lead to cancer.

Although the importance of the NIEHS grantees' discovery that bis chloromethyl ether is a potent carcinogen is unquestioned, the ramifications of this discovery did not end there. The ultimate benefit of the NIEHS-supported research on bis chloromethyl ether lies not only in the fact that a dangerous compound and industrial situation were identified, but also in the fact that other Federal agencies were alerted to the possibility that other halo ethers could be potential human health hazards.

Until a short time ago, it appeared that bis chloromethyl ether is an occupational health problem and not a general environmental health problem for the entire population. This is because almost the entire production of the compound is utilized by the corporation manufacturing it; very little is sold outside the plant.

After the NIEHS had alerted other governmental agencies to the potential hazard of other halo ether compounds, however, the Environmental Protection Agency recently was able to identify several chloromethyl ethers in river water in several places. These compounds were present in discharges from chemical plants in the vicinity and, subsequently, were detected in municipal drinking water. Investigations into the implications of this finding are now receiving high priority and effective steps are being taken to limit exposure and save lives.

It is impossible to determine at this time how many people have been exposed to bis chloromethyl ether or its related compounds. At least 3000 men are known to have been exposed in industrial situations. Other Federal agencies and the industries will be keeping these men under surveillance as a group with above average expectancy of developing cancer.

In addition, the NIEHS grantees at NYU are following up their original work by conducting a retrospective study of cancer of the lung among industrial workers exposed to bis chloromethyl ether since 1948. Records of exposed workers in five companies in which the compound was manufactured or used extensively are being collected from industry files. Mortality and cancer incidence will be determined through Social Security records and death certificates and will be related to the magnitude and duration of exposure in order to develop a clearer understanding of the possible hazard of the material to man.

Coal Dust

Quantitative differences in chemicals which can be extracted from bituminous coal from Pennsylvania and Utah mines may help explain the problem of pneumoconiosis (a chronic fibrous reaction in the lungs to the inhalation of dust).

Dr. Raymond Suskind, an NIEHS grantee at the University of Cincinnati, has conducted extensive studies on the pulmonary effects of coal dust and the role of specific components of the dust in the causation of pneumoconiosis. His laboratory's studies show what appears to be a relationship between the quantity of nickel in the coal dust and the incidence of the disorder.

The most striking chemical differences between the Pennsylvania and Utah coal are in nickel and iron contents, with the Pennsylvania coal being substantially higher in both. The incidence of pneumoconiosis in miners in Pennsylvania is significantly higher than in Utah miners. Moreover, experiments with cultured cells show that extracts from Pennsylvania coal are substantially more toxic than those of Utah coal, a quality which parallels the nickel content in both cases.

Although these studies must be viewed as only suggestive, other published data on nickel indicate that this metal is highly toxic by all routes of administration in living animals and to all types of cells with which it comes into direct contact. It appears, therefore, to warrant more intensive study, particularly as a pulmonary hazard.

Mutagenic Agents

Evidence accumulated through several decades leaves little question that another type of health hazard exists. That is the genetic hazard

from many of the products and by-products of technology and of a substantial number of natural chemical agents. There are yet no adequate methods to determine directly the level of genetic damage produced in populations inadvertently exposed to these mutagenic agents, those capable of producing inheritable changes.

Concern over this deficiency was heightened by the recent discovery that the nitrofuran derivative AF-2, which has been used extensively as a food additive in Japan since 1965, is possibly one of the most potent mutagenic compounds ever discovered. This points out dramatically the need to develop precise and rapid methods to screen chemicals prior to their release, and better methods to monitor the population and evaluate the extent of genetic damage produced in individuals as a result of accidental exposure to mutagens.

Concerns with the AF-2 problem are two-fold. First, we do not have adequate methods to determine whether significant levels of genetic damage have been produced in either the somatic or the germ cells of exposed persons. Second, even though we could look for an increased frequency of chromosome damage in cultured blood samples from exposed persons, we do not have suitable control groups with which to compare the findings. This is because virtually the entire population of Japan has been exposed to AF-2 because of its addition to several Japanese food staples. Thus, we can not ascertain whether the aberration levels are due to AF-2 or represent the spontaneous level found in the Japanese population.

The problem with AF-2 emphasizes the necessity and value of studies by NIEHS researchers of the effects of environmental agents on genetic information in both somatic and germ cells. Dr. Frederick J. deSerres and other scientists are particularly concerned with agents that may cause long-term genetic effects, those that would appear only after a long lag time.

Currently, they are developing a simple assay to monitor the human population for gene mutations. They are attempting to find whether a chemical known to be mutagenic in experimental organisms can produce mutations in man and how these effects can be measured. Test systems that can be used on man, in preference to animals, are needed to determine chromosomal damage and gene mutations.

Because there is growing conviction among scientists that genetic disease probably contributes to inherent susceptibility to other disease and may indeed be causal in some types of cancer, these researchers are also attempting to stimulate other research and by seeking areas of expertise abroad. They now are collaborating with three laboratories in Germany, which are working on new and better methodology for detecting genetic damage, and with scientists in England and Japan. They are beginning a similar cooperative program with scientists in the Soviet Union.

In addition to the work by its intramural scientists, the NIEHS is also supporting—particularly through its grants to Environmental Health Sciences Research Centers—an epidemiological approach to problems in environmental mutagenesis. Through concerted effort, it is hoped that significant strides can be made in the area of environmental mutagenesis.

Aerosols

The widespread use of the aerosol "spray can" dispenser has resulted in another potential human health hazard, including the potential for interfering with certain heart functions.

The spray dispenser has made available a great variety of complex materials in respirable form. While the acute toxicity of many of the active ingredients in such dispensers has been investigated, little is known about the chronic toxicity of these compounds, or the toxicity of combinations of these compounds and the inert ingredients of the pressurized cans.

In studies by Dr. Robert Drew of NIEHS, ex-

Rabbit, in plastic hutch, is prepared for experiment to test aerosol pesticide synergists.

posure of rats, rabbits, and hamsters to aerosols of aluminum chloride-hydroxide, a constituent of virtually all deordorant sprays, have produced increases in lung weights and damage to air passages in the lungs. Some of the fluorocarbons (Freons), which are the propellant gases and solvents in aerosol sprays, have been shown to depress certain heart functions, such as the contractility of the normal heart muscle. In addition, toxicity studies in progress indicate that certain chemicals in widespread use may be highly toxic to animals with pre-existing heart disease or respiratory imbalance.

Polychlorinated Biphenyls (PCBs)

The ability of edible marine species to detoxify or excrete the polychlorinated biphenyls and related chlorinated pesticides may determine whether such animals can continue to be used as a safe food source for humans.

The PCBs are a class of industrial chemicals contaminating this country's waterways. Dr. James Fouts and other NIEHS scientists have been studying these chemicals to determine how they produce the toxic effects.

Marine species are the preferred test systems for two reasons. First, pollutants entering the marine environment are frequently taken up by marine species (both fresh and saltwater) and are highly concentrated. These species are often important as foods, and may expose humans to toxic chemicals. Second, a marine species frequently is particularly sensitive to pollutants, and often predicts a human hazard. By studying the effect of the pollutant on the affected species, NIEHS researchers get advance information on possible human health effects.

What may be a relatively dilute concentration of pollutant in water can be concentrated in marine organisms later used by man. Knowing how fish and other species handle foreign chemicals, and the interactions among them, is important. For this reason, NIEHS scientists all studying the effect of exposure to one xenobiotic, or foreign chemical, on the subsequent toxicity, uptake, storage, metabolism, and excretion of other xenobiotics. In this way, they can learn which organisms will store which poisons and future problems like the concentration of mercury in tuna fish, an acute problem some years ago, can be avoided.

NIEHS studies have shown that the PCBs are not concentrated in most of the edible tissues in

Marine species sensitive to pollutants give information on potential human hazards.

fish and seafood. For example, the muscle masses of the lobster are very low in PCB or DDT content, but the organs of excretion concentrate and store the pollutants. Thus, by discarding organs of excretion, we could retain most of the lobster for human consumption. But these compounds may still be a hazard in shellfish, such as oysters and clams, where the organs as well as the muscle masses usually are consumed.

Asbestos

The problem of how to protect ourselves from hazards associated with compounds that seem essential to our everyday living is sharply apparent in the case of asbestos.

Few other minerals are so much a part of American life as asbestos. Impervious to heat, asbestos fibers are fashioned into fireproof clothing and theatre curtains and such common household items as noncombustible drapes, rugs, pot holders, and ironing board covers. Mixed into slurry, asbestos is sprayed onto girders and walls to provide new buildings with fireproof insulation. It is used in floor tiles, roofing felts, in most plasterboards and wallboards, plaster and stucco, and many paints and putties.

This "mineral of a thousand uses" is probably present in some form in every home, school, office building, and factory. Used in brake linings and clutch facings, in mufflers and gaskets, in sealants and caulking, asbestos also is a component of every modern vehicle, including spacecraft.

At first regarded as relatively harmless to man, it has gradually come to be recognized as the

probable cause of several human disorders. One is asbestosis, a fibrosis or a fine scarring of the lung, which leads to difficulty in breathing, poor oxygen absorption, and poor carbon dioxide removal. This hazard was identified in the early 1900's and recognized as a separate condition in the 1920's.

A second hazard is the development of a previously rare tumor called mesothelioma, a malignant tumor which occurs on the surface of the lung or abdominal cavity. There is not, as yet, a useful treatment for this lethal cancer which was first identified as being related to asbestos exposure in the early 1950's.

In the mid-1950's, the association between asbestos and carcinoma of the lung was developed. Somewhat later, NIEHS grantees at Mount Sinai School of Medicine, New York, found that workers exposed to asbestos who smoke cigarettes heavily are eight times more likely to develop carcinoma of the lung than workers exposed to asbestos who do not smoke.

A fourth biologic effect of asbestos is found in the demonstration by these NIEHS grantees that there is a three-fold increase in tumors of the gastro-intestinal tract among asbestos workers.

The important point about all these effects is that they usually take 20 to 30 years to develop. It has been estimated that out of the approximately 5 million American men and women—workers in asbestos plants, insulation workers, construction workers, steamfitters, carpenters, tile setters, and the like—who daily breathe in large quantities of asbestos fibers, up to 40 percent may die as a result of that exposure. We do not know yet with certainty whether mesothelioma, or other cancer, results only from heavy exposure or whether prolonged low-level exposure will be equally injurious.

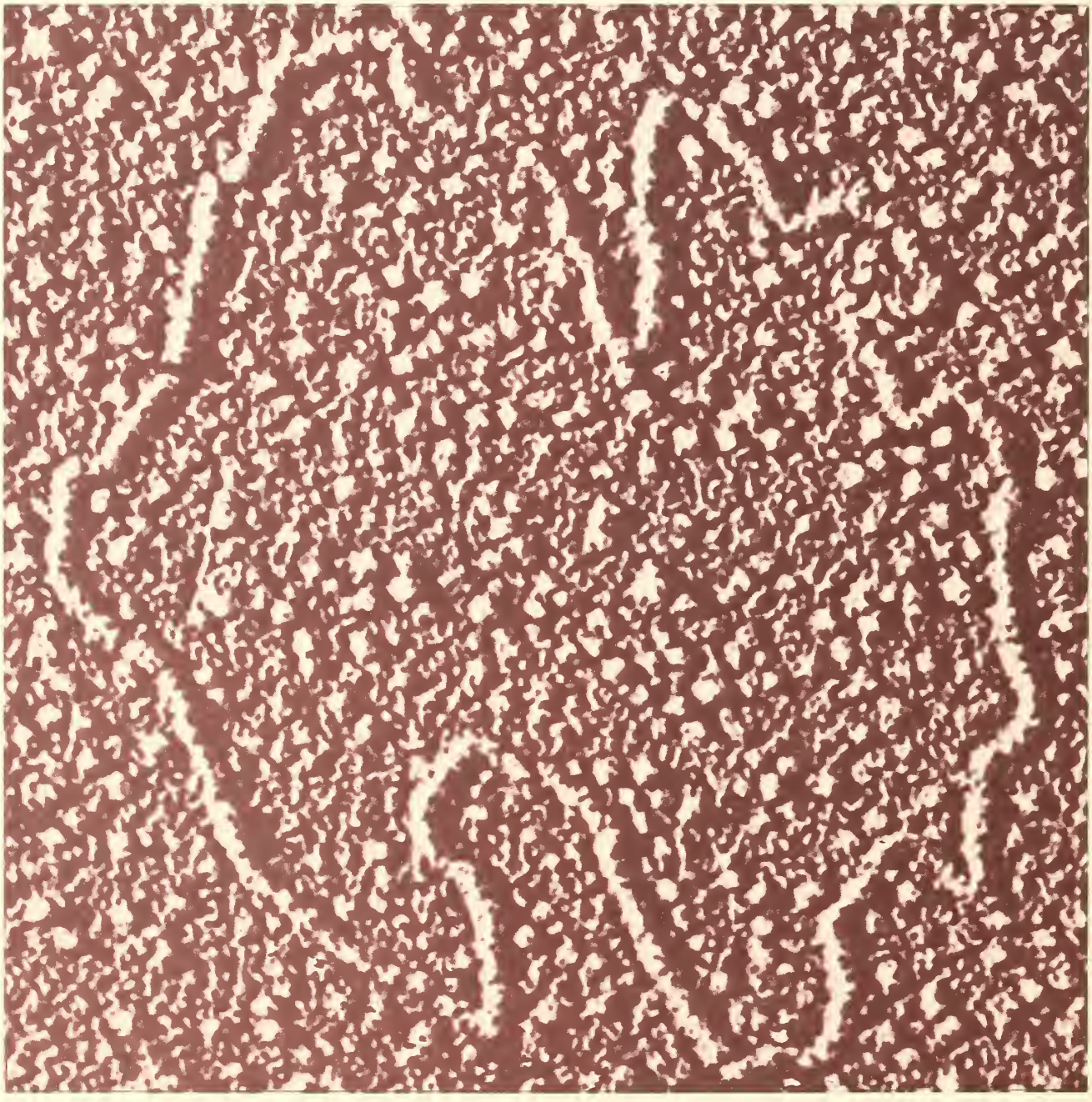
Shipyard workers in the British Isles and in Europe who were building ships in the mid-1930's now are showing an increase in mesothelioma. These men did not work directly with asbestos but rather were down-wind to those applying asbestos insulation to the heating pipes and boilers. This increased incidence of cancer was first reported in about 1970—35 years after initial exposure. In the United States, ship building intensified in about 1940 when we became involved in World War II, and approximately 2½ million workers were exposed to asbestos as were their colleagues in the British Isles and the continent. They now are at risk of developing mesothelioma.

The asbestos hazard would be serious enough if the workers were the only group at risk, but asbestos dust pollution also occurs in situations not related to the work place. Asbestos fibers can be found in the lungs of every adult who lives in large urban areas such as New York City, because the air supply in many buildings is contaminated as it passes through asbestos-lined spaces. There is cause for concern about these large populations, because little is known of the effects of such prolonged, low-level exposure.

NIEHS-supported research, however, is attempting to identify the dose responsible for asbestos-induced disease, thus paving the way for application of appropriate controls. In addition, over the next five years. the NIEHS is investing approximately $2.9 million to support an Environmental Health Sciences Research Center which will concentrate on asbestos research. This center has been established at Mount Sinai School of Medicine under the direction of Dr. Irving J. Selikoff, a pioneer in asbestos epidemiology. It was to Dr. Selikoff, incidentally, that the Environmental Protection Agency recently turned for recommendations when confronted with asbestos contamination of drinking water in Duluth, Minnesota.

Scientists at the center already have developed capabilities for accurate quantitative work. These capabilities will advance understanding of the effects of microparticulates to which man is exposed throughout his life.

Ring-like structure of mitochondrial DNA (mtDNA), similar to that of bacteria, has led to speculation that mitochondria once may have existed as independent organisms but entered into symbiotic relationship with more advanced cell forms. (Electron micrograph by Dr. Jerome Vinograd, California Institute of Technology).



Cellular and Genetic Aspects of Disease

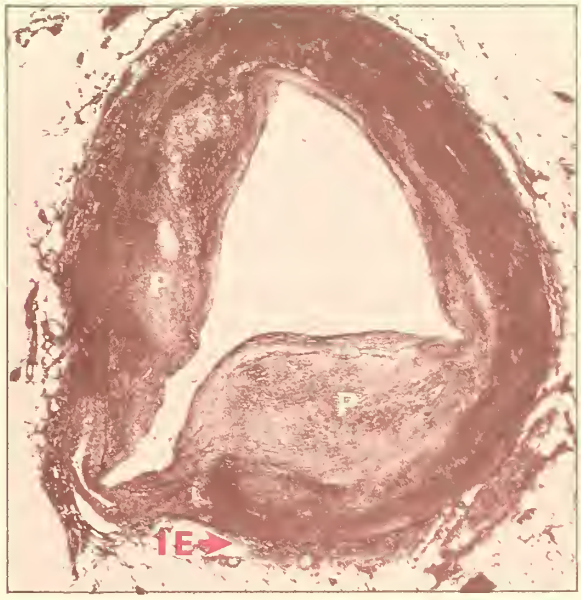
The National Institute of General Medical Sciences, in supporting the research of cell scientists and geneticists, among others, often is in a position to foster work of potentially widespread and direct interest to the categorical, disease oriented Institutes. Just as those studies focussed on a specific disease process often illuminate on unexpected aspect of the normal functioning of the organism, so can studies of normal cell function give a profound, new insight to the mechanism of diseases. The following brief reviews of the results of such research are presented as examples of the interplay among the apparently diverse fields of genetics, cell biology, and pharmacology, on one hand, and the clinical problem areas of cancer, vascular disease, and drug toxicity on the other.

Atherosclerosis: A Benign Tumor?

Atherosclerosis is a vascular disease that develops gradually with age in most people; some individuals and some families, however, show a greater propensity to develop the disease. The lining of the arterial blood vessels shows localized areas of overgrowth of collections of smooth muscle cells, collagen, and cholesterol and other lipid materials. These lesions or plaques may become enlarged enough to obstruct blood flow and cause a stroke or a coronary heart attack in some patients. In others, the weakened vessel may rupture, causing internal bleeding. Fortunately, for the majority of affected persons the process is silent, causing no visible or detectable disease symptoms. Even so, the prevalence of these lesions is so widespread that their origin is a matter of great concern. In one view, it is supposed that cholesterol and other blood lipids when in high concentration may penetrate the vessel wall and, acting as a local source of irritation, cause the lesion to develop.

By contrast, as a result of recent experiments on tissue from healthy and atherosclerotic vessel walls, Dr. Earl Benditt of the University of Washington feels the atherosclerotic lesions may be non-malignant tumorous growths that may be caused by viruses or chemicals in the environment, acting on the cells of the blood vessel walls of genetically susceptible individuals. Dr. Benditt reached this conclusion by extending and applying discoveries of basic scientists in genetics and cell biology to human disease.

The activity of an intracellular enzyme, glucose-6-phosphate dehydrogenase, can be easily measured in very small tissue samples. This enzyme exists in at least two, closely related forms or isoenzymes which can be separately identified. Since the genes determining these isozymes are located on the X chromosomes (two of which are carried by women), women may have either one or both isozymes, depending upon their inheritance. It is hypothesized that one of the two X chromosomes in each female cell is suppressed at random dur-



Atherosclerotic coronary artery near branch point (x 20). IE = internal elastic membrane separating plaque from arterial media. P = fibrous plaques.

ing embryonic development, so that a woman who inherited both isozymes from her parents will show a fine mosaic pattern of cells, some having one isozyme and some the other. By examining and assaying small samples of atherosclerotic plaques from affected females, Dr. Benditt almost always found one or the other isozyme in each sample, but not both. This strongly suggested that each plaque is essentially of "monoclonal" origin, derived from a single cell which had somehow been caused to proliferate. This pattern was in contrast to assays of normal artery walls, where even the tiniest samples would contain a fairly even mixture of the two isozymes (from the mixtures of the two cell types). A total of 30 plaques and 130 samples of normal artery walls from 10 females have now been studied in this fashion.

In addition, there were other specific char-

acteristics of the disease. Histological examination reveals that plaques occur in specific sites near the branching of vessels and on certain vessels, which also appear to be areas where there is a rapid proliferation of the vessel wall cells. Dr. Benditt's groups believes that enhanced cell turnover, possibly in some people the result of high blood cholesterol levels, increases expression of a selective advantage in cells transformed by another agent, such as a chemical mutagen or a virus. Evidence has been obtained that hypertension also leads to increased cell proliferation. It is well known that hypertension predisposes to coronary artery atherosclerosis.

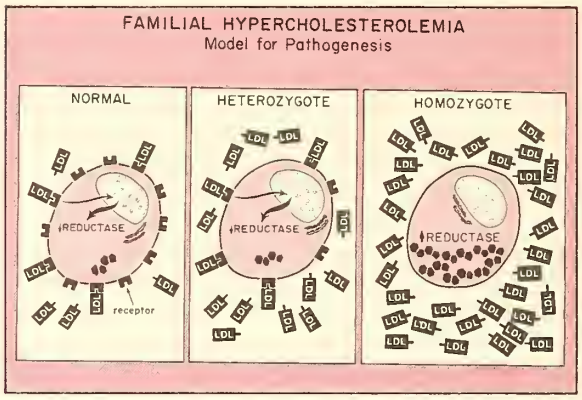
The observations by Dr. Benditt raise the possibility that atherosclerosis has more specific causes than high blood cholesterol levels and may in fact be due to agents such as chemical mutagens or viruses, derived from the environment, acting in a susceptible host.

Cholesterol Synthesis and Heart Disease

It is generally agreed that a high blood cholesterol level can play an important role in the genesis of heart and vascular disease, as previously described. Indeed, one rare affliction (termed the homozygous form of dominant familial hypercholesterolemia) is characterized by extremely high cholosterol levels in the blood and by coronary vascular disease that usually results in the affected person's death before age 30. A basic biochemical defect which results in these high cholesterol levels has now been identified by Dr. Joseph Goldstein of the University of Texas in Dallas, and can be demonstrated in tissue cultures from the skin of such patients.

Ordinarily the rate of production of a key cellular enzyme needed for synthesis of cholesterol is reduced when there is sufficient cholesterol-containing lipoprotein in the circulation. This process, a form of "negative feedback control", appears to be lacking in the cells of these affected patients, who therefore continue to produce large quantities of the enzyme, leading in turn to excessive cholesterol synthesis and high blood levels.

Work by Dr. Goldstein and his colleagues has shown that the primary genetic abnormality is a defect in the cell surface. Normally the event signalling the cell to turn off enzyme production is the binding of the blood lipoproteins to receptor molecules on the cell's surface. It was



Schematic illustrates how lack of receptor sites for low density lipids (LDL) on cell surface of persons homozygous for familial hypercholesterolemia results in inability of their cells to suppress enzyme (HMG CoA reductase) which acts in biosynthesis of cholesterol. Excessive synthesis and high plasma cholesterol levels result.

found that the cells of patients with severe hypercholesterolemia are almost completely lacking in this cell surface receptor.

Tests for the genetic predisposition toward high blood cholesterol levels can now be developed, and suitable preventive regimens can be prescribed early in life. In addition, new insights have been gained on how enzymes are regulated, and how lack of regulation can produce disease.

Human Enzymes and Lung Cancer

The term "human genetic polymorphism" denotes inheritable differences in the structure or function of any molecular component of the body that might distinguish one person from another. Perhaps the best known of these inherited variances are the major blood types, the A, B, and O, and the Rh factors, but many others are recognized and countless more undoubtedly exist in view of the thousands and thousands of different proteins present in the human organism. In general, the great bulk of such variations appear to be not harmful, but knowledge of their characteristics may under certain circumstances assume medical significance, as is the case with blood transfusions based on blood typing.

Dr. Charles R. Shaw of the University of Texas, Houston, has been studying aryl hydrocarbon hydroxylase (AHH), a polymorphic enzyme system that modifies a wide variety of hydrocarbon chemicals, including many drugs, insecticides, and chemical carcinogens, The activity and amount of this enzyme increases when the

white blood cells are exposed to the chemical agents. This is a common phenomenon known as "enzyme induction." The genetic polymorphism found in the AHH system is relative to the increase of enzyme activity after exposure to such agents. For example, after exposure of the white blood cells, the enzyme activity of some people only doubles (type AA); in other people (type BB) it quadruples, while in a third, intermediate, group (type AB) it triples. This variation in "inducibility" is inherited in a simple mendelian fashion. In other words, all children from families in which one parent is type AA and the other is type BB will be found to be type AB.

Dr. Shaw has also shown that persons with the intermediate (AB) or high (BB) inducibility type of AHH enzyme system are far more likely to develop lung cancer than those of genetic constitution AA. Thus susceptibility to this cancer is in part inherited. He feels the AHH enzyme system converts potential cancer-producing chemicals in tobacco smoke or polluted air into active forms, and people with higher induced levels of the enzyme run a greater risk from exposure to these agents.

Dr. Shaw is now perfecting a simple blood test to detect the genetic differences in AHH inducibility. This should enable persons with a greater genetic predisposition toward lung cancer to take precautions to avoid smoking and exposure to other carcinogens.

Vitamin C and Drug Detoxification

It has been recognized that most therapeutic drugs are metabolized in the liver to less active compounds in a process called detoxification. New research now indicates that vitamin C (ascorbic acid), previously thought unrelated to drug metabolism, plays an important role in this process. This work by Dr. Vincent G. Zannoni and colleagues at New York University Medical School is part of a larger study of hereditary variations that modify drug toxicity, which is directed by Dr. Bert N. La Du, now at the University of Michigan.

For this study, guinea pigs were chosen because, like man, in their evolutionary development they lost the ability to synthesize ascorbic acid and are dependent on dietary intake of the vitamin. It was found that when animals were placed on low vitamin C diets they lost their usual capacity to metabolize drugs, and consequently there was a buildup of administered drugs in the blood and toxic side effects developed. The effects of drug toxicity could even be observed in newborn and weanling animals receiving the recommended dietary doses of 1 to 2 milligrams of vitamin C per day to prevent scurvy.

If additional doses of vitamin C were fed to the animals, their rate of drug metabolism was increased accordingly. In general the rate of detoxification was proportional to the ascorbic acid concentration in the liver, which in turn was dependent upon the amount of vitamin in the diet. Thus, it was demonstrated that the amount of dietary vitamin C needed by guinea pigs for adequate drug detoxification may be well above the dietary need, as measured by the classical test of scurvy prevention.

In related experiments, young guinea pigs (2 to 3 months old) were first given a vitamin C deficient diet for 10 days to deplete the liver stores, and then fed high amounts of the vitamin. While the animals reaccumulated ascorbic acid in their livers within 2 to 3 days, their full capacity to detoxify drugs did not return for another week. This time lag suggested to the investigators that vitamin C participates indirectly in the detoxification process. It was further suggested that it probably acts by stabilizing and maintaining the specialized properties of the endoplasmic reticulum membranes of liver cells, which show structural alterations as a result of vitamin C deficiency. In addition, one of the membrane components essential for drug metabolism, the enzyme cytochrome P-450, was found to be reduced in quantity.

Whether a similar relationship between vitamin C and drug detoxification exists in man remains to be determined. If so, perhaps man requires higher vitamin C intake than usually recommended. However, this will clearly depend upon current and future investigations in man to determine whether a high drug detoxification ability would in fact be beneficial.

Ribosome Assembly

If the information contained in the genes stored within the cell nucleus is an "encyclopedia" of the total genetic potential of an organism, the messenger RNA, which carries the information out of the nucleus to its site of use, must be considered as a disposable copy of a given article. It can also be considered as a sort of "tape" with instructions for making specific proteins needed at certain times and

places in the life history of an organism. Ribosomes contained in each cell are "machines" that read this tape and, along with a suitable supply of amino acids, metabolic energy, and other accessory factors, translate the information in the tape into functional proteins.

Although microscopic in size, the ribosome is a highly complex organelle, consisting of two different subunits, each composed of a large molecule of ribonucleic acid and 20 to 30 different protein molecules. As with any complex machine, one can study the ribosomes by carefully disassembling them into their component parts. Features of ribosomal proteins and nucleic acids are being investigated extensively by a number of NIGMS grantees. However, an even greater skill is needed to reassemble such a complex structure from its parts. More importantly, if the reconstituted ribosomes re-exhibit their normal activity, one has demonstrated that all the necessary pieces have been identified and is then in a position to learn more about the detailed inner workings of these genetic "machines." This can be done by replacing one component protein or RNA with another, and looking for any changes in function that accompany this exchange of parts.

Thus, the successful reconstitution of ribosomes by Dr. Masayasu Nomura of the University of Wisconsin is an exciting scientific accomplishment. The process is roughly analogous to the assembly of a fine watch, in that it can only be done sequentially: first, several specific proteins must be allowed to interact with the RNA molecule before others can be be added; other particular proteins can only be assembled into the ribosome near the end of the process.

The importance of this research is indicated by the fact that ribosomes constitute as much as a quarter of the dry weight of some cells. Since they are complex structures each consisting of 50 to 60 macromolecules, ribosomes may well be engaged in other important functions in addition to protein synthesis. For example, one of the enzymes participating in phospholipid synthesis in the bacterium (*E. coli*) also happens to be a ribosomal component and thus may play a role in coordinating the synthesis of lipids and proteins. Further studies can be anticipated in which reconstitution of mixtures of mutants and normal ribosome components will play an important part.

Dr. Nomura is perhaps best able to sense the significance of this achievement. He believes it should be possible to reproduce in the laboratory all the assembly events that occur in living organisms. The achievement of such a complete system would represent a necessary step toward comprehensive understanding of the biogenesis of organelles, and eventually, of the more complex behavior and genesis of cells.

Genetic Material in Mitochondria

The formation or biogenesis of mitochondria is a matter of great interest to cell scientists because mitochondria (small structures in the cytoplasm of cells) have been found to contain their own hereditary material, DNA, in the circular form that is characteristic of the DNA found in bacteria. Scientists would like to discover which of the proteins and enzymes of the mitochondria originate from the information carried in the mitochondrial DNA (mtDNA) and which originate from the information stored in the cell nucleus (nDNA), the source of information for the synthesis of most of the body proteins. This knowledge would be of considerable importance for the design of antibiotics and drugs, for example, and for the understanding of genetic disease.

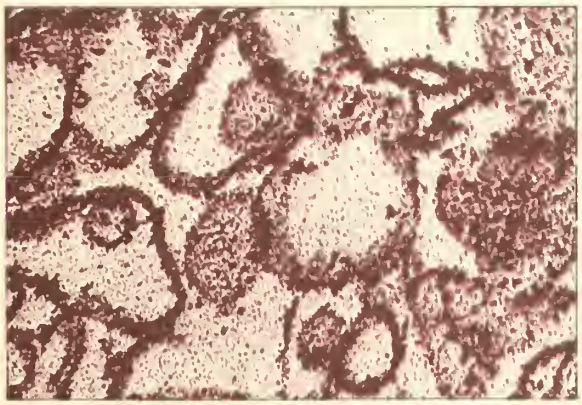
Dr. Gottfried Schatz of Cornell University has found a unique system to study the process of mitochondrial biogenesis. When yeast cells are grown in the presence of air or oxygen they contain mitochondria that appear normal. When grown anaerobically, or in the absence of oxygen, they do not, but instead have tiny particles called "promitochondria." Dr. Schatz believes these are the precursors of mitochondria, because when the cells containing them are later placed in oxygen, the particles develop into mitochondria. If the oxygen is again removed, the mitochondria shrink and become promitochondria again, losing many of their enzymes in the process, including the cytochromes important for cell respiration. However, these degenerate organelles retain some function, since the enzyme ATPase is still present and active, and exhibits the same response to drugs as in normal mitochondria. This, therefore, is the elegant but simple system being used by Dr. Schatz to study the formation and assembly of the hundred or so different enzymes, coenzymes, "coupling factors" and components that make up mitochondria.

The first experiments have shown that in the presence of oxygen the promitochondria can

synthesize 10 proteins ranging from 11,000 to 94,000 in molecular weight. Since synthesis of these materials is completely inhibited by acriflavin, a drug that blocks mitochondrial protein synthesis but not nuclear synthesis, it appears that each of the 10 proteins are true mitochondrial translation products as coded by the mitochondrial DNA. Subsequent studies have added supportive evidence.

Genetic Origins of Cytochrome Oxidase

In a closely related research project, Dr. Alexander Tzagoloff of the New York City Public Health Research Institute is studying the formation of a single mitochondrial enzyme to trace its origin either to mitochondrial DNA or to nuclear DNA. The enzyme in question is cytochrome oxidase (or cytochrome a_3) which is responsible for a final step in cell metabolism involving reactions with oxygen. Dr. Tzagoloff has found that cytochrome oxidase is composed of seven subunit proteins, each of small size, with molecular weights ranging from 7,500 to 28,000, and each apparently different. He has purified five of these proteins and is working on the remaining two. Using a chemical agent called cycloheximide, which specifically blocks the formation of proteins from nuclear genes, Dr. Tzagoloff has shown that three of the seven subunit proteins are synthesized from information contained in mitochondria DNA—including the largest subunit protein, which comprises part of the "active site" of the enzyme. The remaining four protein subunits are derived from nuclear DNA. Clearly, cytochrome oxidase represents a complex structure, made of seven separate protein chains, of which three are derived from genetic information contained in the DNA of the mitochondria and four from information stored in the cell nucleus.

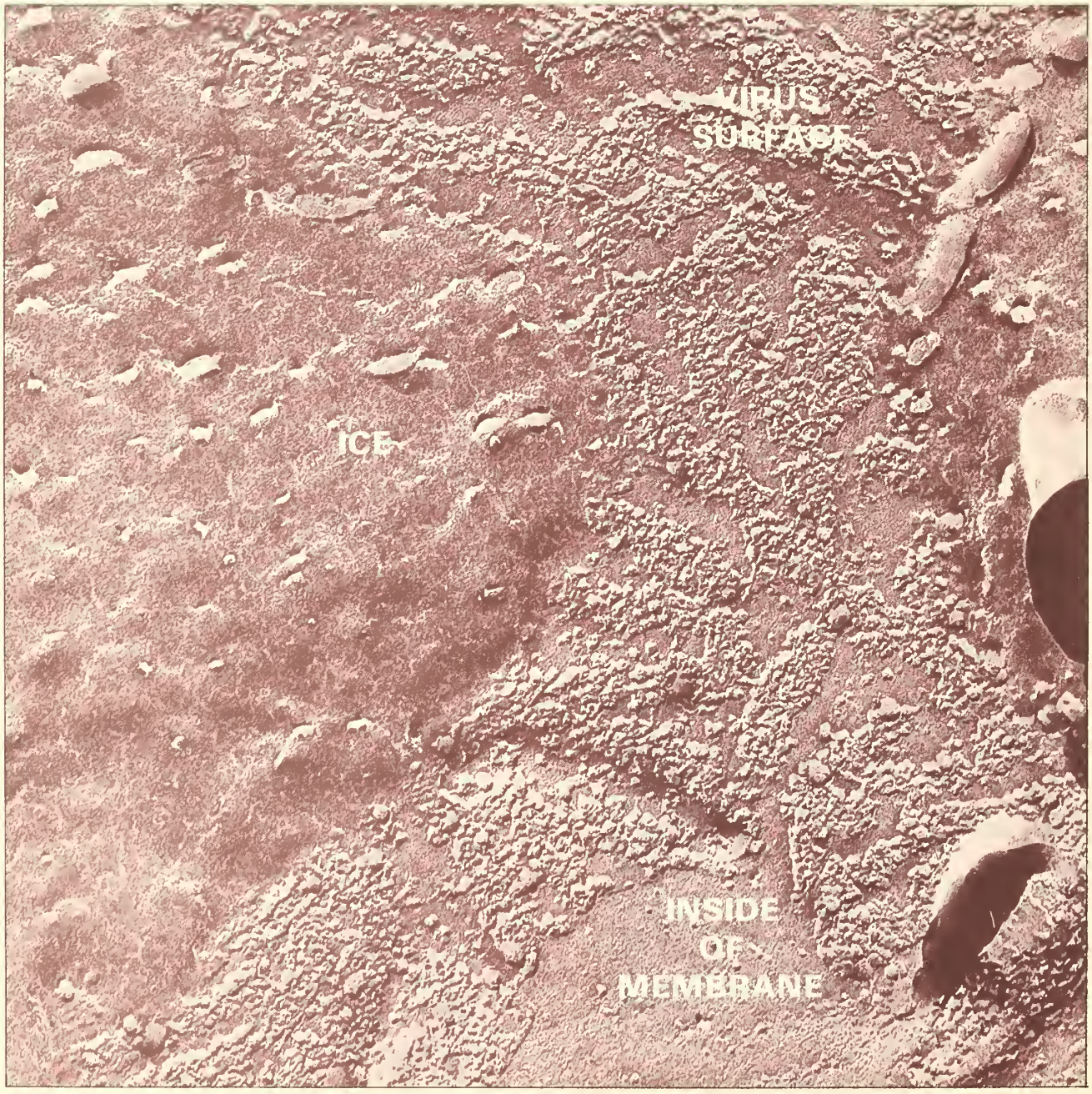


Electron micrograph shows purified cytochrome oxidase particles reconstituted as vesicular membranes with the addition of phospholipids.

The synthesis of this single enzyme from two cooperating, but separate sources of genetic information—the mitochondria and the nucleus—is a matter of great curiosity to scientists. How is formation of the two sets of protein subunits coordinated in space and time, and how are they all assembled? Is one set of protein subunits incorporated into the structure of the internal membrane of the mitochondrion prior to the other, or do they unite before entering the membrane surface? Answers to these and related questions will have considerable significance in our understanding of those the events in our ancient biological lineage when the precursors of mitochondria were somehow incorporated into other cells, destined to form the basis for all higher forms of life, including man.

The National Institute of Neurological Diseases and Stroke

Using new techniques of rapid freezing of cell and immunolabelling, scientists halted viral activity and captured panorama of virus budding out of cell membrane in this electronmicrography. Virus is a measles variant isolated from childhood neurological disease, subacute sclerosing panencephalitis. (x 11,500).



One of the most significant advances in the neurological sciences over the past decade is the discovery that a virus or virus-like agent is directly responsible for the transmissibility of certain degenerative central nervous system (CNS) disorders. This finding could lead to further understanding of the cause of amyotrophic lateral sclerosis and multiple sclerosis.

Further Evidence of Slow Virus Transmission

Six fatal degenerative diseases of the central nervous system have been shown to be caused by slow or atypical viruses that remain in the body months or years before any clinical expression of a disorder. Most of these discoveries have been made in the last few years by scientists of the National Institute of Neurological Diseases and Stroke (NINDS).

Four of these progressive neurological disorders occur in man (kuru, Creutzfeldt-Jakob disease) subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy) and two (scrapie and mink encephalopathy) are found only in animals.

In 1959, an NINDS scientist, Dr. D. Carleton Gadjusek, observed similarities between scrapie (a disease of sheep known to be transmissible in select breeds) and kuru (a fatal disease found among a primitive people in New Guinea). This led in 1962 to the launching of a research effort in which specimens of brain tissue from kuru victims and from sheep with scrapie were injected into several species of subhuman primates.

Early in 1965, one of eight chimpanzees inoculated with tissue taken from a kuru victim began to show symptoms of neurological disease. Within three years all eight had kuru symptoms.

By 1967, the disease had been passed from chimpanzee-to chimpanzee three times in the classic test for infection. The theory that kuru in man came from a slow virus had been proved. A wide hunt for similar agents in other diseases was instituted.

The disease which bears the closest resemblance to kuru is Creutzfeldt-Jakob disease (CJD). In 1968, NINDS scientists successfully transmitted CJD from a patient to a chimpanzee. This experiment was later repeated, and the disease was transmitted further from chimp-to-chimp and to other animals.

Two recent reports provide additional evidence for the transmission of Creutzfeldt-Jakob disease. Israeli scientists have found a remarkably high incidence of CJD among Libyan Jews (31.3 per million) relative to that in other Jews. Iraqi Jews and native Israelis, for instance, each had an incidence of 1.0 per million.

What may be the first evidence of person-to-person transmission of CJD was recently reported by investigators at the College of Physicians and Surgeons of Columbia University. A 55-year-old man died of pneumonia after a

Pre-adolescent kuru patients, Kuru Research Hospital in New Guinea.

2-month history of progressive failure of memory, with loss of muscle control, involuntary movements, and abnormal muscle spasms. A corneal transplant from this patient was given to a 55-year-old woman. Subsequently CJD was diagnosed from the autopsy of the donor. The recipient of the cornea developed CJD symptoms 18 months later. She died 8 months after the onset of the symptoms and the autopsy on her body showed the characteristic changes of CJD.

Noting the rarity of CJD, the investigators consider it unlikely that its occurrence in both the donor and the recipient of the graft was coincidental. They suggest that proof of the cause-and-effect relationship requires other studies.

In an attempt to provide such proof, NINDS scientists have transplanted the corneas of a capuchin monkey with experimental CJD to two healthy monkeys. This procedure was performed on April 2, 1974, and the grafts have taken well. It may be many months before any signs of disease are observed in the recipient monkeys.

In unpublished data from this laboratory, the investigators suggest a possible link between the high incidence of CJD in Libyan Jews and

the possible person-to-person transmission reported by the Columbia scientists. They note that sheep's eyeballs are considered a gastronomic delicacy by Moroccans and Libyans. Scrapie, a disease of sheep, has clinical and histopathological features similar to CJD.

The NINDS group suggests that ingestion of eyeballs of sheep harboring the scrapie agents might possibly lead to the development of CJD in susceptible persons and thus account for the high incidence in Libyan Jews.

Basic Findings Spur Paraplegia Research

Paraplegia is another area of urgent NINDS research activity. Injuries to the spinal cord are growing in frequency and are now a main cause of death and paralysis among persons in the prime of life. Since World War II, great progress has been made in rehabilitating spinal cord injury patients but, despite many attempts, there has been little success in reversing the effects of the injury itself.

Scientists have known for some time that hemorrhagic destruction of tissue in central areas of the spinal cord can produce paraplegia. Until recently, however, the scientists thought this destruction of the cord occurred immediately, as a direct result of the trauma.

A few years ago, an Institute grantee demonstrated in experimental animals that the hemorrhage may not occur for a number of hours, indicating that its cause may be something other than immediate disruption or laceration of the intrinsic circulation. The grantee, Dr. Jewell L. Osterholm, Hahnemann Medical College and Hospital, Philadelphia, suggested that the cause may be a dramatic increase in the concentration of norepinephrine or a closely related substance in the injured area of the cord.

Norepinephrine is a neurotransmitter in the brain and peripheral tissue. As this chemical agent is released from nerve-fiber endings, it can stimulate or inhibit other nerve cells to influence physiological processes.

Dr. Osterholm observed that increased liberation of norepinephrine (one of a group of compounds known as catecholamines), produced constriction in blood vessels of the spinal cord. The resulting loss of circulation and tissue destruction at the site of injury precluded further regeneration. However, by injecting alpha methyl tyrosine, an agent that interferes with the synthesis of norepinephrine, Dr. Osterholm reduced damage associated with cord trauma. This treatment produces the best recovery to date from spinal cord injury in experimental animals.

These findings led to a hypothesis that the catecholamines are critically involved in production of some of the functional and anatomical abnormalities resulting from spinal cord trauma. Although other investigators have not yet been able to confirm this observation completely, it has provided a valuable framework for new understanding of microcirculation, the biochemistry of cord metabolism, and the activity of catecholamines.

To take advantage of these promising developments, the NINDS is supporting a national research effort on acute spinal cord injury in centers at Yale University, Ohio State University, St. Joseph's Hospital and Medical Center, Phoenix, Ariz., New York University, and the Medical University of South Carolina.

The fresh insights emanating from these centers and from basic studies by NINDS scientists are helping to dispel the pessimism traditionally attached to acute cord trauma and to offer paraplegia patients the hope of possible therapeutic success.

Enzyme Replacement Therapy

Recent technology for unraveling the metabolic chemistry of the cell, has identified a substantial number of familial neurological diseases as errors in metabolism.

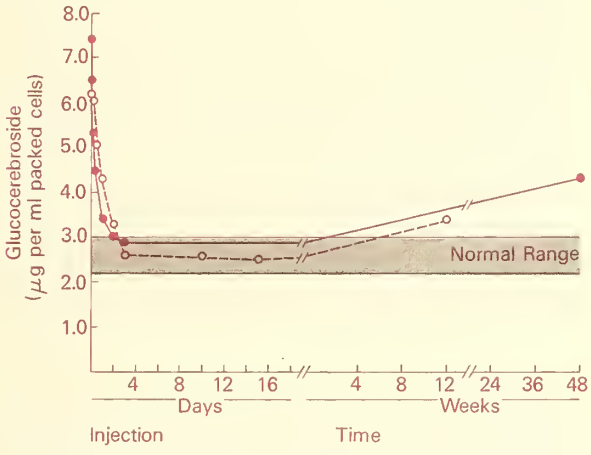
Beginning with the identification of the enzyme defect in Gaucher's disease in 1965 by NINDS scientist Dr. Roscoe Brady, investigators have been able to pinpoint the specific defect in each of the 10 currently recognized disorders of lipid or fat metabolism in the nerve cell. These hereditary conditions, which include Fabry's and Tay-Sachs diseases, are marked by the accumulation of fatty substances called sphingolipids in various tissues and can cause a wide range of physical illnesses, mental retardation, and early death.

Practical developments have resulted from these discoveries. Rapid and inexpensive tests have been devised to diagnosis victims. Procedures have been developed to identify familial carriers of the genes that cause the disorders. And, because of these advances, genetic counseling can be performed with accuracy.

NINDS researchers are now pursuing a new

therapeutic approach that may open a new era in treating inborn errors of metabolism. This is called enzyme replacement therapy. Dr. Brady and his associates recently demonstrated in patients with Fabry's disease that replacing the deficient enzyme by intravenous injection of normal enzyme obtained from human placenta can immediately reduce the amount of accumulated lipid circulating in the patient's blood.

This successful therapy to reverse temporarily the effects of the metabolic defect represents



Purified enzyme (glucocerebrosidase), injected into two Gaucher's disease patients, brought reduction of the disease-causing lipid (glucocerebroside) in their red blood cells to normal within 48 hours. Level remained normal or near-normal for almost 3 months.

a significant advance. It is the first beneficial enzyme replacement therapy for a genetic disorder.

Enzyme replacement studies also are being undertaken in patients with Gaucher's disease. Preliminary results are equally encouraging. In fact, it appears that injection of a single high dose of the purified enzyme into these patients may eliminate about 26 percent of the lipid accumulated in their livers. Since it takes about one year for a patient with Gaucher's disease to accumulate this amount of lipid, it is conceivable that treatment might be as simple as an annual enzyme injection.

Isolation and purification of enzymes is arduous and expensive, but NINDS scientists are hopeful that these obstacles will be overcome and that this important health problem can be eliminated.

New Epilepsy Therapy Approved

In 1960 Congress passed and the President signed legislation requiring that new drugs be proved effective as well as safe before they are approved for prescription use. In 14 years, no new drugs for long-term treatment of epilepsy received such approval from the Food and Drug Administration.

The drug carbamazepine (trade name Tegretol), which has proved effective in control of complex partial and generalized tonic-clonic seizures, has now received FDA approval for use as an anticonvulsant. The approval results from the first cooperation between a Federal health research agency and a drug manufacturer in producing data for an FDA evaluation of an epilepsy drug.

Drugs such as diphenylhydantoin (Dilantin), phenobarbital or primidone (Mysoline) control seizures in at least 60 percent of epilepsy patients and partially control seizures in an additional 20 percent. The 20 percent with partially controlled seizures and the remaining 20 percent whose seizures go virtually without control are expected to benefit most from carbamazepine.

NINDS assisted CIBA-GEIGY, Ltd., developers of carbamazepine, in conducting three studies of the drug under research contracts. Clinical data from prospective, double-blind trials at New Castle State Hospital, New Castle, Ind., contributed to the drug's approval. Contract-funded studies, still in progress, are being conducted at Lafayette Clinic, Detroit, Mich., and at the University of Washington School of Medicine.

Although carbamazepine has been used to treat epilepsy in Europe for more than a decade and has been approved in the United States since 1968 to treat the severe facial pain of trigeminal neuralgia, it has been in an investigational status for treating epilepsy.

Epileptic patients in New Castle State Hospital under the care of Joseph T. Brook, M.D., Clinical Director, participated in studies to determine carbamazepine's efficacy in preventing intractable tonic-clonic seizures in a chronically hospitalized population. Drug concentrations in patients' blood were monitored using liquid chromatography or GLC. (GLC is a chemical procedure, recently adapted to epilepsy research, largely at the instigation of NINDS scientists. This procedure measures anticonvulsant drug levels in patients' blood, enabling physicians to prescribe drug doses giving maximum effectiveness with a minimum

of side effects.)

The Lafayette Clinic is studying carbamazepine's effectiveness in controlling complex partial seizures in outpatients. Preliminary data from this trial and from a similar one underway at the University of Washington indicate carbamazepine is also effective in treating this type of seizure.

Dr. J. Kiffin Penry, NINDS, who administered the contract trials and cooperated with CIBA-GEIGY in obtaining FDA approval of carbamazepine, acknowledged that the drug is not a final answer to epileptic seizure control. He emphasized, however, that it is an important addition to the store of therapeutic agents to control seizures in patients who have not responded to previously available medications.

Ear Implant for the Deaf?

Is it possible to open up a world of sound to profoundly deaf persons living in a noiseless and wordless vacuum? Some investigators working on an electrode device to be implanted in the inner ear's cochlea (the organ of hearing) think there may be a chance.

Conventional hearing aids are of little or no help to the approximately 300,000 profoundly deaf persons in this country.

But in the great majority of profoundly deaf, there is at least partial survival of the acoustic nerve which transmits signals from the ear to the brain. About one-third of these persons have partial nerve function.

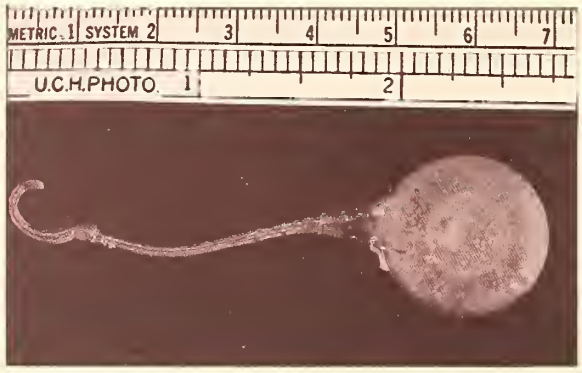
Some scientists feel an implantable hearing device could make use of this remaining function to transmit electrical signals from the ear to the brain.

One of these is NINDS grantee Dr. F. Blair Simmons, head of Stanford University Medical School's Department of Otolaryngology, Stanford, Calif.

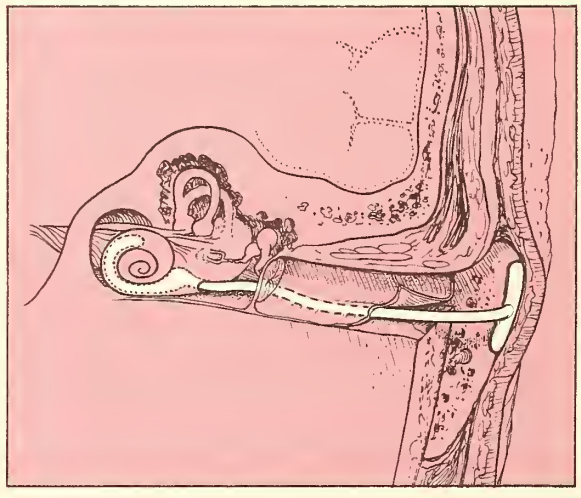
Dr. Simmons and his colleagues are developing a multichannel electrode system they feel will be able to achieve maximum information transfer. Some of the questions they are seeking to answer are these: How much coded acoustic information could be transmitted to the brain? What are persons with implants actually hearing? And what discriminative learning is taking place?

Evidence has accumulated for 40 years showing that stimulation of the inner ear produces auditory sensations in profoundly deaf persons. At least four groups of researchers in this country—three of them receiving NINDS support—are now studying aspects of cochlear implantation.

Experts in the field participating in a meeting with NINDS officials feel there are at least two different approaches to an auditory prosthesis. If the objective is to provide some awareness of general environmental sounds, a single electrode system may accomplish this.



Hearing prosthesis consisting of cochlear implanted receiver and electrode array, (above). Below, receiver with electrode array implanted in cochlea (dotted line).



California ear specialists, who have implanted single electrodes into more than 20 deaf persons, report that these persons are able to appreciate sound—like a doorbell or phone ring—and that the implant assists patients in lipreading by providing cues on rhythm and cadence.

The alternative is to assist the deaf person to achieve improved social communication, including speech. It is not yet known whether a peripherally implanted prosthesis could en-

able the wearer to perceive speech, although scientists feel electrical stimulation of the acoustic nerve by such a device might prevent nerve degeneration.

As a result of the meeting, several important areas of animal research were proposed, including studies to design, develop and test electrodes and stimulators. Research also is needed on human cochlear implants, including criteria for selecting and evaluating implant patients. In addition, the scientists felt, new rehabilitative and educational measures would be needed for patients with an auditory prosthesis.

Hypertension and Stroke Recurrence

The increasing use of drugs to control high blood pressure (hypertension) is accepted as one of the reasons for the gradually increasing survival rates from stroke, and for a gradually decreasing incidence. Many physicians, however, have been uncertain about the safety of antihypertensive drug therapy in patients who already have had strokes. They have felt that drastically lowering blood pressure might be disastrous in a patient whose cerebral circulation already has been reduced. And they have hesitated to interfere with a situation to which the cerebral blood vessels may have gradually adjusted.

These fears should be reduced and standards of therapy improved by the results of a large-scale cooperative study just reported by NINDS grantees. The scientists have found that long-term reduction of blood pressure does not increase the risk of stroke recurrence, and have established a basis for recommending antihypertensive drugs for stroke patients even if they have only moderately elevated blood pressure. The group also has confirmed that antihypertensive drug therapy in stroke patients reduces the risk of congestive heart failure.

The study was the first to provide these types of data from a group of people with mild to moderate hypertension. Patients in this category are often not undergoing any hypertensive treatment.

Ten university medical centers and large hospitals joined in the study, which began in September 1966 and ended in June 1972. A central registry was set up at the University of Michigan Medical Center under the supervision of Drs. S. W. Hoobler and Arthur Ericsson, succeeded in 1969 by Drs. Stevo Julius and John F. Simpson. A medical risk review section monitored the study quarterly to determine any possible events indicating undue risk. Each center set up a separate stroke-hypertension clinic, with an internist and a neurologist in charge. There were 452 stroke survivors with recumbent blood pressures in the mild to moderate ranges (readings from 140 to 220 millimeters of mercury systolic and 90 to 115 millimeters diastolic pressure) observed for a mean period of three years. Half of these received

antihypertensive drugs and half received placebos, inert medication. Drugs used were a combination of deserpidine and methyclothiazide. There were 37 stroke recurrences in the drug-treated group, and 42 in the placebo-treated group. This was not a statistically striking difference, but a clear demonstration of safety, and a good indication that these drugs are beneficial.

Stroke is the third largest cause of death and the leading cause of long-term disability in the United States. Each year about 500,000 Americans are stricken, and about 200,000 die. There are about 2 million stroke survivors in the country, with varying degrees of disability.

An extensive effort is being made to develop surgical procedures and drugs to lessen the effects of stroke. Since brain cells cannot survive an interruption of blood supply for more than a few minutes and can never be replaced, after-the-fact measures probably will never do more than halt continuing brain damage. Prevention thus becomes a more effective goal. The prospect of being able to prevent many thousands of strokes within the next few years

was raised in 1970 when a Veterans Administration study definitely established that drug treatment of high blood pressure can bring about a great reduction in deaths from strokes. The NINDS study provides valuable data to physicians concerned with prevention of second strokes, and promises to result in a further reduction of deaths and disability.

Rubella Yields Viral Infection Clues

How the body recovers from viral infection remains one of the most crucial questions that plagues medical science today. The rubella (German measles) syndrome, a tragic "experiment of nature," may be hastening the resolution of this question.

NINDS scientists, Dr. David Fuccillo and associates, have found that congenital rubella (passed from the mother to the fetus) impairs a growing embryo's cellular immune system. This system is one of the body's three defenses against invading disease. They have found further that reinfection with rubella virus alters this impaired cellular response, and may in fact spur it back into action.

These discoveries pose two very important questions: how does rubella virus destroy cell-mediated immune response? And how does the infant finally rid itself of this virus, which can cause an array of devastating problems from deafness to mental retardation?

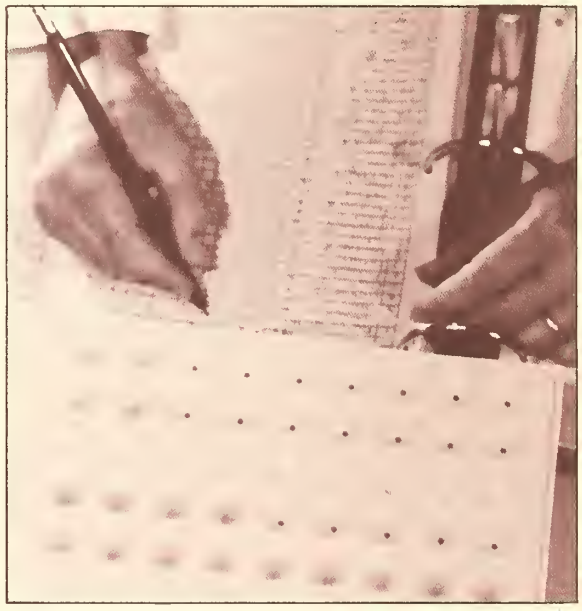
Fortunately, the recently developed rubella vaccine has drastically reduced the incidence of congenital rubella infection. Determining rubella's role in these two processes may, however, be crucial to understanding the action of other congenital viruses which, like rubella, can produce intrauterine infection and consequent severe damage to a developing infant.

When a rubella pandemic swept through the nation in 1964, complete clinical data were available from a unique patient population of more than 50,000 women and their offspring who were participating in an NINDS study of perinatal factors influencing health.

Babies in this study population who were born with congenital rubella were found to have antibody (a substance produced by the body to combat invading foreign organisms, such as viruses). Yet even in the presence of antibody, it was discovered, the disease persisted long after birth. This showed two things: one, the antibody system was not damaged by the virus; and two, the system was not instrumental in curtailing the infection.

Studies on interferon—a second defense system—showed it too was intact. Interferon is a protein produced by a virally infected cell which instructs neighboring cells to block viral entry by changing their molecular machinery.

The scientists found, however, that rubella had impaired a third system, called cell-mediated immunity (CMI). This system utilizes slow re-



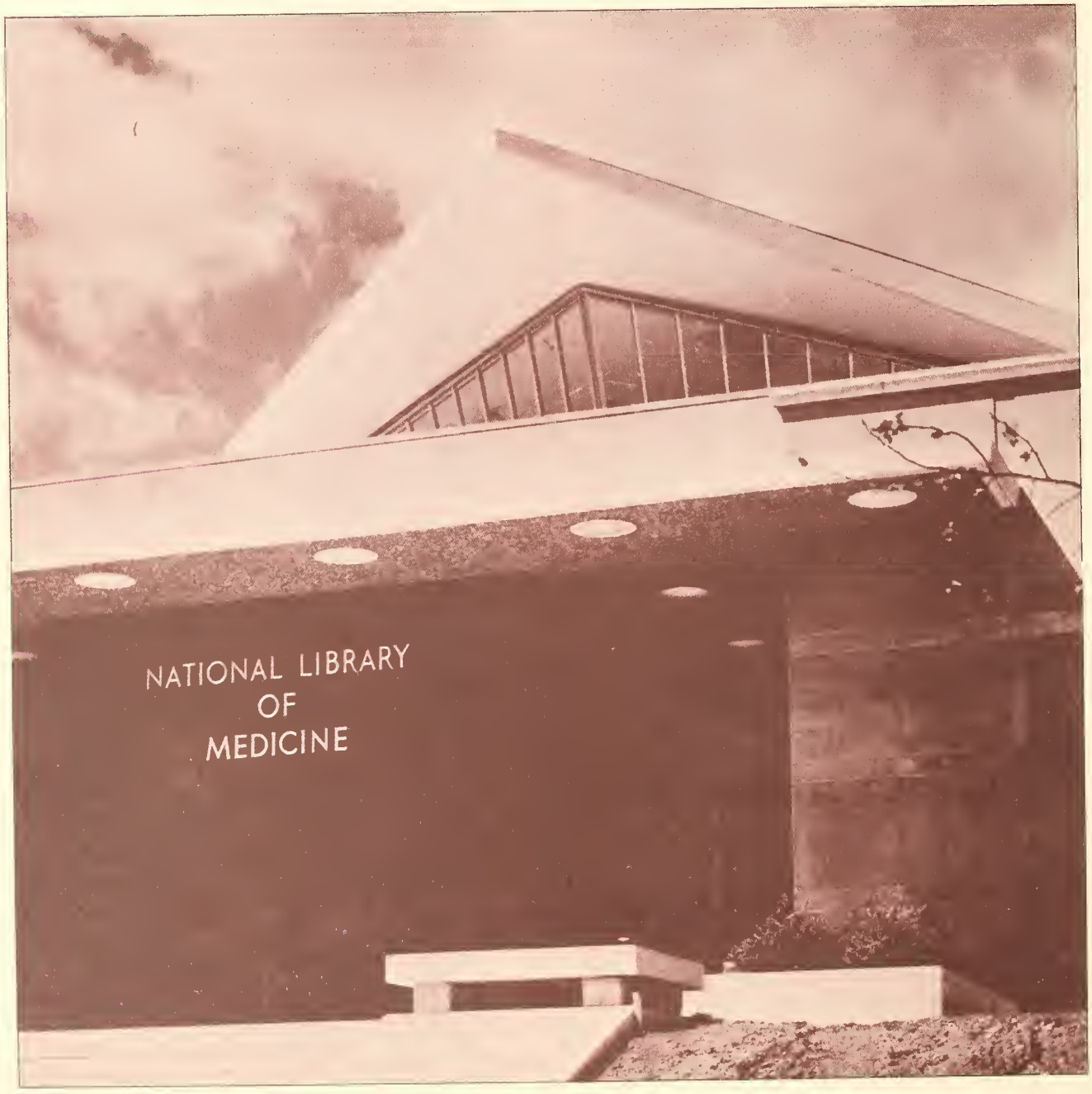
acting lymphocytes, (a type of white blood cell involved in immune reactions) called T-cells, which originate in the thymus gland and attack virally infected cells.

Using a procedure known as a lymphocyte transformation test, NINDS researchers found that rubella had almost totally annihilated the T-lymphocytes. The scientists are now seeking to learn how rubella destroys the T-lymphocytes and what role, if any, CMI may play in helping the body finally slough off the virus. There is evidence to suggest that rubella may not destroy all of the T-lymphocytes in the thymus, and those which remain and reproduce may later be stimulated to fight off the virus.

This evidence comes from studying two congenitally infected children who became reinfected with rubella and subsequently developed high levels of T-lymphocytes. It is not known whether reinfection alone, or whether some other factor, stimulated production of

the T-lymphocytes.

Determining whether—and if so how—cell-mediated immunity resolves viral infection could lead to effective treatment. Similarly, determining how the virus impairs cell-mediated immunity may lead to methods of preventing initial viral infection.


NATIONAL LIBRARY
OF
MEDICINE

Medline—Instant Access to Biomedical Literature

An obvious but often overlooked fact is that without effective communication the progress of biomedical research is impeded and the time lag between discovery and application of new knowledge lengthens. The National Library of Medicine, with well over a century's experience in serving American medicine, has pioneered in applying technology to the transfer of medical information to reduce this gap.

One new way for helping the biomedical community to get the information it needs is via MEDLINE. MEDLINE is an on-line computer-based system to retrieve references to biomedical journal articles. It is designed to help health professionals find out easily and quickly what has been published recently on any specific biomedical subject.

The "on-line" feature of MEDLINE means that a physician, researcher, medical librarian, or other health professional can "converse" with the computer—specifying a series of questions until a list of references on his precise topic of interest is retrieved. The average time for a complete search is less than 15 minutes.

MEDLINE is accessed by typewriterlike terminals in several hundred hospitals, medical schools, and research institutions throughout the country. These terminals are connected by telephone to a central computer at the National Library of Medicine in Bethesda, Md. It is this same computer which is the heart of MEDLARS (Medical Literature Analysis and Retrieval System), used to prepare *Index Medicus* and other publications containing references to the medical literature.

The MEDLARS data base contains over two million references to articles from about 2,000 of the world's leading biomedical journals dating back to 1964. MEDLINE (an acronym for MEDLARS On-Line) contains over 500,000 of these references published since January 1972.

The computer recognizes some 9,000 medical subject headings—from abattoir to zymosan—that can be combined to search on a topic. In addition to the subject headings, the MEDLINE user can search by an author's name, words in the title of an article, publication date, language, a specific journal, or a combination of any of these elements. A listing of all references meeting the desired search parameters is printed out at the terminal. The printout contains author, title, and journal source for each reference. It is also possible to print out the medical subject headings used to index each retrieved article—a sort of "telegraphic abstract."

MEDLINE is an outgrowth of a search capability originally built into MEDLARS. Previously, search requests were received by mail, prepared for the computer by a specialist, and the

Terminal connected by telephone to NLM computer gives health professionals throughout the U. S. access to banks of bibliographic information.

results mailed back to the requester. The entire process took several weeks, depending on the vagaries of workload and mail delivery. MEDLINE, with its ability to respond instantaneously to a request, is infinitely more powerful. Available to users some 80 hours a week, Monday through Friday, MEDLINE is now processing searches at the rate of over 250,000 per year.

MEDLINE services in the U.S. are coordinated by eleven Regional Medical Libraries, each responsible for a designated geographical area. These libraries are also vital links in a growing medical library network engaged in exchanging books and journal articles on loan. Journal articles identified in a MEDLINE search can be requested through a local library which in turn has access to a Regional Medical Library for material not in the local collection. The National Library of Medicine backstops the entire network.

The ability to retrieve not telegraphic, but actual abstracts, will be added to MEDLINE beginning late in 1974. This feature will be of great help in ensuring that references retrieved are, in fact, germane to the user's needs. Growth of the system in terms of number of terminals on the network has slowed, further increase depending in part on enlarged computer capacity. Use of the system in terms of number of searches performed, however, continues to grow dramatically.

Computerized Toxicology Information Available Nationwide

Similar in some respects to MEDLINE is another on-line service called TOXLINE (Toxicology Information On-Line). TOXLINE was developed by a Toxicology Information Program established at the National Library of Medicine in 1967.

Operational since late 1972, TOXLINE is an information retrieval system for health professionals and scientists working in the areas of environmental pollution, occupational health and safety, poison control, pharmacology/toxicology, medicine, and related disciplines. Like MEDLINE, TOXLINE is accessible via a nationwide communication network which connects users to the computer at the National Library of Medicine. A MEDLINE user is not automatically given access to TOXLINE, however; he must subscribe separately to TOXLINE.

By the summer of 1974 over 100 government agencies, academic institutions, and commercial organizations were TOXLINE users. Although the system is available coast to coast, there are fewer institutions using TOXLINE than MEDLINE because of the more specialized nature of the data base. Until 1974, TOXLINE was operated for the Library by a contractor and a charge of $45 per hour of terminal use was levied. In the spring of 1974, TOXLINE was put on NLM's computer and operated in parallel with MEDLINE, eliminating the contractor and reducing the cost to $15 per hour. This is still somewhat higher than the $6 per hour charged for access to MEDLINE.

TOXLINE has several capabilities not presently found in MEDLINE, however. First, its data base contains not only references to the indexed toxicology literature, but, in most cases, the full abstracts representing the article. By 1974, TOXLINE contained some 300,000 entries, including references from *Toxicity Bibliography,* abstracts from *Pesticide Abstracts,* abstracts from *Chemical Biological Activities,* references and abstracts from *Abstracts on Health Effects of Environmental Pollutants;* abstracts from *International Pharmaceutical Abstracts,* and miscellaneous references on the health effects of pesticides.

Another feature of the system is that it permits access not only to the *literature* of toxicology/pharmacology, but also to chemical nomenclature data. This is done through an associated file—CHEMLINE—available to all TOXLINE users. This data base contains the 60,000 chemical compounds uniquely identified by Chemical Abstracts Service (CAS) Registry Numbers in TOXLINE. CHEMLINE contains, in addition to the CAS Registry numbers, the molecular formulae, CAS-approved names, and generic and trivial names for all compounds.

Using a terminal in his own institution, the TOXLINE user (like the MEDLINE user) is able to search the data base by subject heading, author's name, journal title, publication date, language, or a combination of these elements. In addition, however, the TOXLINE user has a "free text" search capability. That is, he is able to search the data base using any word or string of words that might appear in the abstract.

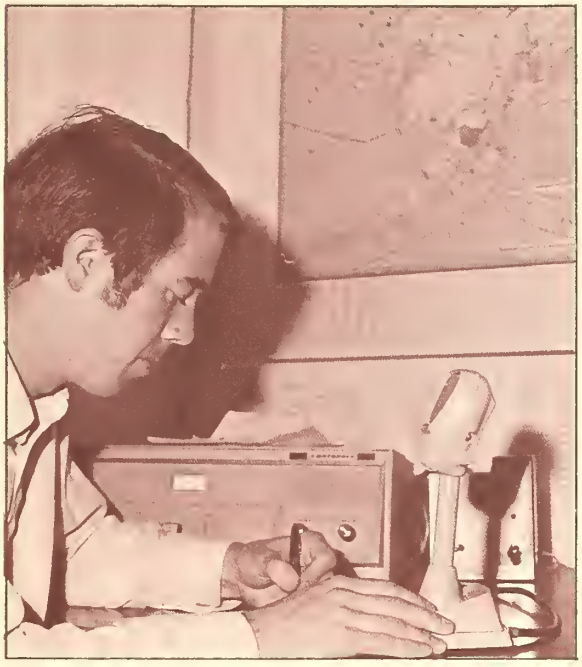
The value of on-line retrieval services to physicians, researchers, educators, and information specialists has been demonstrated by MEDLINE and TOXLINE. This is apparent from the growth of these systems in number of user institutions and number of searches performed. Further expansion is foreseen not only in the U.S. but also in Australia, Brazil, Canada, France, Germany, Great Britain, and Sweden, all of which have made (or are preparing to make) NLM's on-line search services available in their countries.

Medical Communications Via Satellite

MEDLINE and TOXLINE are *library*-oriented information retrieval systems. The National Library of Medicine also sponsors several projects to apply modern communication technology to health care delivery and health education.

The first of these, satellite communication, was introduced to "bush" Alaska in late summer of 1971 through an experimental medical net-

work sponsored by NLM's research component, the Lister Hill National Center for Biomedical Communications, in cooperation with the Health Services Administration and the Health Resources Administration. The National Aeronautics and Space Administration's Applications Technology Satellite (NASA's ATS-1) is used like a tall (22,000-mile-high) tower providing line-of-sight communication to and from each of 26 ground stations located throughout the state. The ground stations,



Public Health Service physician makes daily "house calls" via satellite radio to health aides in remote Alaska villages.

costing about $3,000 each, were installed by the University of Alaska's Geophysical Institute, which manages the network and maintains the equipment.

The experiment involves daily two-way radio communication between community health aides in isolated communities and a Public Health Service physician located at the PHS Hospital in Tanana. Health aides, who after 12 weeks of training provide all primary health care in their communities, are now able to consult daily with a doctor concerning treatment of their patients; formerly radio contact was uncertain and was plagued by periods of "blackout"—no communications—for periods of up to a week at a time.

Each of the aides has a "satellite radio" in her home connected by cable to a nearby antenna. Every day a doctor at Tanana calls the health aides in turn on the single "party-line" satellite radio channel. Health aides may ask the doctor for specific instructions after describing signs and symptoms, or may verify their own diagnosis and treatment. The medical facilities in the villages are still very limited, so that any serious cases must be evacuated. The satellite radio can then be used to arrange for a plane to evacuate such patients.

The communication network has been credited with saving lives in several medical emergencies, such as severe burns from the explosion of a stove and serious complications during childbirth. In each case, the satellite radio made possible immediate step-by-step first-aid instruction for the health aide, followed by prompt air evacuation of the patient.

Although reliable voice consultation enhances the health aide's abilities, in many situations it is not possible through words alone to provide enough information to the physician about a patient's problem. Sometimes a medical decision must be delayed until the patient's condition changes or until he can be flown to a medical center. Delays in diagnosis and unnecessary evacuation could in many cases be avoided if the physician had adequate information—especially visual information. "Telemedicine," now possible in Alaska with a new satellite, the ATS-6, may provide the answer.

The ATS-6, launched from Cape Canaveral on May 30, 1974, is sufficiently powerful to provide medical television in Alaska with simple, relatively inexpensive ground terminals. Using ATS-6, it is possible to provide complete audio/data/video communication links between the nurses and community health aides in two remote villages and the physicians in Tanana and medical specialists in Anchorage. Using the satellite's capability for transmitting high-quality television, the physicians and specialists are able to "see" patients at the two villages and can prescribe treatment and follow the patients' progress.

The experiment using the new ATS-6 will end in 1975 when the satellite will be moved to a point serving India, for education experiments there. The ATS-1 network, already proved successful, will continue to provide voice communication in Alaska.

Interactive TV Network

In another project, the National Library of

Medicine is attempting to demonstrate that two-way interactive television can assist in providing continuing medical education for rural doctors. Television can connect the medical school classroom with small community hospitals and in effect bring busy community practitioners to the university classroom.

Supported by the Lister Hill Center, the New Hampshire/Vermont Medical Interactive Television Network consists of seven institutions in the two states linked by a microwave system. The hub of the network is the Dartmouth Medical School in Hanover, N.H. Three of the institutions on the network, all within a 20-mile line-of-sight radius of the transmitting tower on Mt. Ascutney in New Hampshire, are served by a mobile unit that carries microwave transmitting and receiving equipment, and TV cameras and monitors. On arrival at a site, the van is connected to local power and the site becomes part of the network.

With two-way voice and video communication, the network has become a regular working tool for medical and allied health personnel at the seven locations. Each week, air time is allotted to programs on such subjects as grand rounds, speech therapy, conferences in neurology and neurosurgery, psychiatric consultations, and courses for nurses.

Different types of users benefit from the network. The medical centers use the network as a vehicle for service delivery and for faculty sharing. Local community hospitals derive status, goodwill and needed information as participants, and continuing education for their staffs. General practitioners find that the network offers them a low key, job relevant "break," and an opportunity to test ideas with colleagues at other institutions. Specialists, on the other hand, welcome the opportunity to maintain contact with physicians at other institutions. Nurses, who have had formal courses offered over the network, find that it offers them both knowledge and opportunity for certification.

A survey by a management consulting firm shows that interactive television is an effective medium for providing educational and other services in a rural medical setting—and that for formal educational purposes television can be as effective as classroom instruction. The network also may promote "best medical practice" by affirming practitioner judgments on case management and encouraging more widespread adoption of new practices.

The next two years will be devoted to demonstrating cost benefit to the point where user institutions will assume a substantial portion of the costs.

Computer Assisted Instruction Network

In a time of rising costs of education and pressure to increase medical student enrollment, it becomes more important than ever to devise methods for efficient sharing of educational resources. The most recent of the experimental networks developed by NLM's Lister Hill Center addresses this problem with a network for sharing computer assisted instruction (CAI) systems among medical schools and hospitals.

This project has been spurred by interest in recent years in developing tutorial computer programs with which students can interact using keyboard terminals. Students progress through such a course at their own pace, guided at every turn by the instructions.

In the new network, over 80 institutions have access to a wide range of CAI programs. There are basic science programs for the first two years of medical education, and programs that simulate disease syndromes and clinical encounters appropriate for the second two years. Many a computerized "patient" has "died" as a result of his encounter with a young physician-to-be, who learns thereby the right choices to be made in real practice. There are also programs of interest to nurses, nursing students, optometry students, and staff members of community hospitals.

The instructions available were developed at the Massachusetts General Hospital, the Ohio State University College of Medicine, and the University of Illinois Medical Center, Chicago. Computers at the first two of these institutions are tied into a nationwide communications network so that students in many institutions can use them.

According to reports of the using institutions, about 70 percent of the use is by medical students, the remainder by physicians, nurses, and allied health personnel. Total usage has ranged as high as 2,800 hours a month (there are an average 2.5 teaching sessions per hour). Since there is a tendency for several students to group around a terminal and to interact with each other as vigorously as with the pro-

gram, it is probably conservative to estimate that the network is providing over 15,000 interactive teaching sessions per month.

The experiment is demonstrating that although schools are interested in sharing CAI materials, and students are eager to use them, the present configuration of the network is too costly for the long run. Therefore, NLM is exploring alternative distribution methods, some of which may involve an initial capital expenditure on the part of the using institutions, but

Dermatologist in "consultation studio" at University of Vermont examines a patient in center 65 miles distant via interactive television.

which will result in substantially reduced operating costs.

Economic viability is an important consideration in all the biomedical communication projects the Library supports. Except for such information retrieval systems as MEDLINE and TOXLINE, NLM acts as a catalyst for innovation—a developer and demonstrator of new methods which can then be operated by others.

Finding the Elusive Parathyroid Gland

Early and detailed diagnosis is often the key to effective treatment of important diseases. More patients with chronic diseases could be effectively treated if the afflictions were detected in early stages and the site and extent of involvement precisely determined. Parathyroid disease, an endocrine disorder being diagnosed with increasing frequency in this country, is an example.

The parathyroids are tiny hormone-secreting glands located in the neck behind the thyroid. Parathyroid hormone is responsible for metabolism of calcium and phosphorus in the body. When a tumor or other disorder causes the parathyroid to secrete too much hormone, loss of calcium in the bones results in progressive pain, tenderness, spontaneous fractures, muscular weakness, and abdominal disorders.

With surgery to remove the diseased tissue, the prognosis for patients with hyperparathyroidism is excellent. However, the size and varying location of the glands make them difficult to locate, and surgery therefore may not always be successful.

Health screening tests that detect elevated calcium levels in the blood before other symptoms of hyperparathyroidism appear are now in use. This means that the disease is diagnosed earlier, when the glands are even smaller and more difficult to find at surgery.

Recently, investigators at the NIH research hospital, the Clinical Center (including staff doctors of the National Cancer Institute and National Institute of Arthritis, Metabolism, and Digestive Diseases), have developed a method of locating the diseased glands that has been accurate in nearly 100 percent of patients with newly diagnosed disease, and over 80 percent of those for whom earlier treatment failed.

The method is called selective venous catheterization and uses a radioimmunoassay test to measure parathyroid hormone in the blood. Difficult-to-find parathyroid adenomas, for instance, are located by looking for increasing levels of the hormone in the veins of the neck and upper chest as the catheter tip gets closer and closer to the malfunctioning gland.

The veins are explored using a fluoroscopically directed catheter in the tributaries that drain the thyroid gland and the surrounding tissues. In these veins, elevations of parathyroid hormone several times the peripheral blood level correlate with the location of the gland or tumor, and suggest whether multiple glands are involved in the overproduction of hormones.

In patients who have undergone prior parathyroid surgery, the vein system is sometimes altered or removed and alternate routes established to drain the area. In such instances, arteriography, a procedure in which a dye or marker is injected into the arteries, is performed to locate the alternate pathways.

The procedure takes several hours, but enables physicians to fix the precise location of the gland and forewarn surgeons of any difficult situations.

Non-Invasive Determination of Ventricular Function

Other Clinical Center investigators have developed an improved method for diagnosing and studying heart disease. Working with staff of the Division of Computer Research and Technology and the National Heart and Lung Institute, they have adapted a computer to help interpret nuclear medicine tests. The advantage of this method, called ECG-gated scintigraphic angiocardiography, is its disclosure of precisely what happens as the heart ventricle expands and contracts.

A radioactive Technetimum-Human Serum Albumin tracer is injected into a vein and spreads uniformly through the bloodstream. A scintillation camera records the radiation given off as the blood is pumped through the heart ventricle. As the ventricle fills and empties a series of images or "pictures" of the heart action is displayed.

In theory, a complete picture could be made from a single heart pumping cycle, but since only a small amount of radioactive tracer is used, the amount of radiation given off in one cycle is too small to produce a complete picture. Accordingly, the images from as many as 1,000 to 2,000 heart cycles are superimposed until a reliable picture is accumulated.

This process uses a small computer system that records the scintillation camera data as well as an electrocardiograph reading of the R-wave that marks the heart's electrical activity just before each ventricular contraction. The R-wave clues the computer to overlay the scintillation data on the picture from the previous cycle. The result is a sequence of 10-milli-

second pictures representing the entire cycle. The series can be analyzed for ventricular function and evaluated for efficiency of the pumping action.

The procedure takes 20 to 30 minutes and can be performed on an outpatient basis with very low radiation exposure and no discomfort. It can be repeated often to trace progress of disease or treatment and, unlike radiographic catheterization studies employing direct injection of material into the ventricle, does not alter the patient's cardiovascular status.

The Division of Computer Research and Technology

Mathematics and computer science may not, to the casual observer, seem to be the stuff from which improved health care and successful medical research derive. For years, however, the computer has been used as an effective tool in the arena of medical inquiry, and the attendant disciplines of mathematics, physical science and engineering have supplied theories and practical solutions to problems faced by physicians, biologists, and chemists.

Cell Interaction

For instance, the disciplines of molecular genetics and biochemistry have taught us how biological materials are synthesized. We know that genetic material directs the synthesis of large molecules but we have only a primitive idea of how these molecules then form multimolecular cellular structures. Similarly, we do not know what phenomena underlie the ability of a cell to adhere to an artificial surface or another cell to form multicellular aggregates, and why, in some cases they do not aggregate.

Some of these fundamental questions asked traditionally by biochemists and immunologists, are now legitimate concerns of physicists and mathematicians. At the Division of Computer Research and Technology at NIH, Dr. Adrian Parsegian, a physicist, is interested in finding what makes cells stick together in some cases and what makes them repulse each other under different conditions. It has been theorized that cells interact on a molecular level to make contact and that perhaps some biochemical reaction in the cell membrane causes the cells to aggregate. Dr. Parsegian believes some cells do not aggregate as a direct result of long-range forces acting between cells.

Collaborating with Dr. Parsegian are Drs. Peter Rand and Dennis LeNeveu of Brock University in Ontario, Canada. Using a theory Dr. Parsegian propounded with Dr. David Gingell of London, which served to predict what would happen in actual experiments, the Canadian scientists attempted to measure intercellular repulsive forces. They used artificial lipid membranes, believed to be a good model for real membranes, which ordinarily adhere to each other in a stack-like array. As forces were altered on the stack, the scientists observed spaces forming between membranes. They measured the changes in force that caused one membrane to repulse another and found the changes confirmed the theory.

The studies conducted were systematic, combining the disciplines of physical chemistry, theoretical physics and biophysics. The scientists hope that once it is understood what controls membrane contact—what is normal or abnormal cell interaction—the physical characteristics and forces on the cell membrane may be modified to prevent aggregation of living cells in abnormal disease states.

A Neural Modeling System

Elsewhere in the Division, Dr. Erik Pottala has been studying neural behavior and has accumulated information on the neuron (nerve cell) and its responses from the published medical literature. He was interested in designing and building a "hardware" model of a neuron—that is, an electrical system wired so that it reacts to stimuli very much the way a living nerve cell would.

Dr. Pottala designed his model and programmed a computer to "stimulate" it—to control various inputs to the model which would then react in a physiological way. An example of the model's usefulness was in an explication of the "stepping" up phenomenon. It had been theorized that when a person takes a step, there is probably a small neural network at the spinal level that is stimulated either centrally or peripherally to activate extensors (muscles which extend or straighten a limb) and flexors (muscles which bend the limb) alternately. This spinal neural network, along with other internal stimuli, functions to give walking its specific characteristics.

To confirm this theory, Dr. Pottala and colleagues at the University of Maryland used the model to simulate the motor neurons connected to the various muscles used in stepping. He then programmed a minicomputer to supply the inputs to the model—the internal stimuli from various muscles, from tendons and from the spinal neuron net itself. By modifying the input neural patterns, the results or output from the model was made to match what has been experimentally determined in living animals. He was thus able to make the model react as an animal would.

Modeling techniques of this sort may prove valuable for clinicians who can use it to predict the neural behavior of an organism, and

also to control the conditions of their experiments. Models are also used to excellent advantage in teaching.

Technology for Medical Decision-Making

A different kind of problem is addressed by Judith M. S. Prewitt, mathematician, and Douglas Reece of the Division's staff. Taking analysis of patient medical data as their province, they have designed a system which allows a physician to store, retrieve, and analyze medical data from a computer quickly and easily.

The system is interactive. That is, by using a typewriterlike terminal linked to a cathode ray tube display (or television tube) the scientist may converse with the computer in a language that is understood by both. The language is the heart of the system. It is a limited subset of English which is suitable for dealing with the problem under investigation. The language is close to everyday English, not an abstruse programming language the researcher must first laboriously learn.

Since the scientist is to use this "mini-language," the computer must also be provided with a way to deal with it. It must have a grammar and a set of computer programs which allow it to scan, parse and interpret the queries put to it. In addition, it must also contain the data base which the scientist is anxious to explore and which the computer searches to answer his questions.

The system is divided into three components called DECIDE, PEEP and GRAPH. DECIDE explores the data base and analyzes the numerical and literal data. For instance, DECIDE has been used on files containing cardiac data of patients who are candidates for aortic valve surgery. By using the computer to probe the data base, physicians have been able to predict which patients will be reasonable risks.

The second component, PEEP, edits, explores, transforms and displays pictorial data. GRAPH is an interactive display system for producing two-dimensional or three-dimensional displays on the cathode ray tube. All three components have been used for characterizing cancer cells of the epithelium or lining of the urinary bladder. The computer peruses microscope slides of the pathological tissue and objectively and quantitatively detects abnormalities present.

Mathematicians and physical scientists collaborate with bioscientists in the NIH environment. There is nothing inherent in their disciplines which makes them medical researchers. The application of their disciplines to biomedical problems, however, has resulted in new insights and techniques directed toward solutions.

Nonsurgical Therapy for Gallstones

The possibility of dissolving gallstones in the body is closer now than ever because of research at the Mayo Clinic, Rochester, Minn. Fifteen years of concentrated study on bile acids by Dr. Alan F. Hofmann may bear fruit, as use of a pharmacological substance to dissolve gallstones approaches feasibility.

The compound, chenodeoxycholic acid (chenic acid), is a natural bile acid in man. Dr. Hofmann's work with Dr. Johnson L. Thistle, another Mayo Clinic researcher, may eliminate the necessity for high-risk patients to undergo cholecystectomy (gallstone surgery).

A controlled clinical trial, utilizing chenic acid dosage, was conducted with 30 men and 26 women at the Mayo General Clinical Research Center which is supported by NIH's Division of Research Resources. After six months, more than half of the patients showed reduction in gallstone size. Subsequent X rays revealed the gallstones had disappeared in 13 of the patients.

Gallstones are caused by a cholesterol oversaturation in the bile. Cholesterol is kept in solution by two other components of the bile, lecithin and bile acids. When the ratio of cholesterol to lecithin and bile acids is too great, gallstones form. If the concentration of bile acids can be increased, the cholesterol again will become soluble and the gallstones will dissolve. The chenic acid technique attempts to achieve proper equilibrium between the components of the bile.

Dr. Hofmann feels that factors determining the saturation of bile are still not thoroughly understood, and his research is continuing. He is internationally recognized as a proponent of chenic acid therapy and was chairman of a workshop on the subject in Freiburg, West Germany.

The concept of cholesterol gallstone dissolution by oral dosage of chenic acid will be evaluated, with controlled toxicity studies, in a national cooperative trial funded by the National Institute of Arthritis, Metabolism, and Digestive Diseases to establish ten gallstone study centers throughout the country. Approximately 100 patients will be treated in each center.

Dr. Leslie J. Shoenfield—another pioneer in the field and Director of Gastroenterology of the Cedars-Sinai Medical Center in Los Angeles—has been appointed coordinator of the study.

"The status of chenic acid at present may be compared with that of the first contraceptive pill," says Dr. Hofmann. "The important event that has occurred is that the feasibility of a medical approach to gallstone dissolution has been demonstrated. As far as I know, from published literature, no other compound has induced gallstone dissolution."

The Degu—New Research Animal

Biomedical research on thymic function and cataract formation in the human body may be advanced because of the development of a dimunitive South American rodent—the degu—as a laboratory animal model.

Two physiological quirks in this species indicate the degu may prove most useful. The animals are born with two anatomically separate thymus glands. In addition, upon reaching adulthood, a high percentage of the animals develop cataracts.

Originally housed at the Massachusetts Institute of Technology, the 500-degu colony is now flourishing at the University of Vermont in Burlington. Dr. David K. Boraker, immunologist, is in charge of the colony, largest in the United States, which is supported by a Division of Research Resources grant.

The double thymus phenomenon is of interest to immunologists who are attempting to decipher the function of the thymus, the master organ which in early life sets up the immune defense mechanisms for children. Researchers at the University of Vermont feel that the anatomical location of the double thymus in the degu makes it far more accessible for research than in the traditional animal model—the mouse.

The thymus in humans and in most other mammals is a single structure located in the upper chest cavity. The degu is consistently born with a cervical thymus (located in the neck), and a mediastinal thymus (located in the upper chest cavity). The cervical thymus can be removed easily without harming the animal.

Sometimes referred to as a trumpet-tailed rat, the degu weighs about 7 grams at birth and grows to nearly 300 grams. It lives 3 to 5 years, yields two litters per year of five newborns, on

the average, at the end of a 90-day gestation period.

The discovery of sugar cataracts in adult degus was of interest to Dr. Jin H. Kinoshita, chief of the Laboratory of Vision Research, National Eye Institute. The Vermont researchers, in collaboration with Dr. Kinoshita's group, found the degus apparently have a metabolic disorder in sugar metabolism which results in the precipitation of cataracts and may be analogous to diabetes.

A separate colony with a genetically defined strain of degus with high incidence of cataracts may be established at the University of Vermont.

The Division of Research Services (DRS) provides support for intramural research at the National Institutes of Health. It concentrates on the unique requirements that develop as research investigators' plans and experiments evolve. The Division's critical mix of professional workers includes microbiologists, veterinarians, nutritionists, biomedical engineers, glassblowers, technical artists, environmentalists, medical photographers, research librarians, pathologists, chemists, and animal caretakers, to name a few. They work in close collaboration with bench scientists and administrators, but often also contribute skills and talents in independent research and development to provide needed products or services.

Animal Models of Human Disease

One of the most specialized demands upon the Division is for well-defined, reliable models for research in diseases and abnormalities threatening human health. Many of the animal models now available were developed at NIH, and breeding stock is provided to medical researchers throughout the world. DRS geneticists are developing several new models which promise to speed access to new knowledge. They include a mouse strain to assay whooping cough vaccine, rat strains for studying high blood pressure and research on eye disease, rabbits uniquely bred for immunological research, and gerbils for investigating epilepsy.

One remarkably promising animal model is the Patas monkey, in which humanlike disease patterns exist. Patas monkeys originate in the grassy plains of Sudan and Ethiopia. A joint effort of the Division and the National Heart and Lung Institute is defining this monkey for studies on atherosclerosis. This degenerative disease of the arteries is characterized by deposits of fatty substances which narrow blood passages, with ultimate hardening and loss of elasticity, and is often associated with heart attack and stroke. The fatty substances, cholesterol and lipids, may be easily and accurately measured in Patas monkeys and may be altered by controlling the diet.

DRS veterinarians and nutritionists have recorded and mapped the fatty deposits resulting from varied diets. Patterns of the deposits are demonstrably similar to the patterns in human atherosclerosis. Recently the scientists developed a diet which resulted in no evidence of the dangerous deposits after one year of study. Other diets produce dense fibrous patches, known as plaque, in the blood vessels, similar to those found in man, and this is enabling investigators in the National Heart and Lung Institute to demonstrate progressive stages of the disease.

As the monkeys were studied in this project they were examined daily. Dr. David K. Johnson, a DRS veterinarian, observed unusual dental problems apparently related to diet and recognized this for potential investigation by the National Institute of Dental Research (NIDR). As a result, the Patas monkey also has become a valuable model for studying dental plaque, the deposits that support bacteria causing tooth decay.

Experimental diets also produced hyperplasia in gum tissue, an abnormal multiplication of normal cells. Collaborative research by the Division and NIDR also is under way in this area. In another study, the relationship between *Streptococcus mutans* infection in severe dental disease and endocarditis, infection of the heart lining, is being investigated.

The "Nude" Mouse

Another important function of the Division is production of small animals, primarily rodents, which comprise the majority of all animals used in biomedical research. The Division maintains more than 125 different strains and stocks of small animals, many with unique genetic components. This repository, recognized as an international resource, is truly a "living library" for research.

One outstanding mutant recently discovered is the "nude" mouse. It derives its name from the most noticeable of its many unusual traits, lack of hair. The most important of its abnormalities is absence of a thymus gland, the master organ which establishes the body's first immune defense system. Studies revealed that the thymus does not develop in the nude mouse embryo and the remaining, almost invisible, rudimentary gland provides no immunity. Absence of the thymus drastically shortens the life span and is an interesting oddity in itself. When considered in the context of our knowledge of the human immune response system, it becomes an important research tool.

As the nude mouse is extremely susceptible

to disease in a conventional environment, complex, expertly applied techniques are required to maintain breeding colonies and to rear the young in an infection-free setting. These developments also aid other research.

One immediate application will be in cancer research. Used as a host, the nude mouse makes it possible to transplant and grow normal and tumorous tissue from other species including humans. For the first time, human solid tumor tissue will grow in a laboratory animal, maintain the characteristic tissue structure and at least some of the functions. The Division is cooperating with the National Cancer Institute so the full potential of this strain can be realized.

Selective cross breeding was undertaken by DRS geneticist Dr. Carl T. Hansen to establish the nude gene in 18 inbred strains for different immune response studies. Some strains will be identical except for a specific hereditary trait. It usually takes two to three years to develop such a strain.

Because it lacks resistance to infectious agents, the nude mouse is also used to study the hepatitus virus. Research with the causative agents of this serious liver disease had been hampered by the lack of a suitable animal model. A collaborative study of hepatitis using the nude mouse is in progress with the National Institute of Allergy and Infectious Diseases.

Medical Instrumentation—Focus on Ultrasonics

Biomedical engineers in the Division aid research in several ways. One effort is directed against the difficult problem of medical diagnosis when inspection of the inner organs is restricted. X-ray photographs can provide pictures of internal skeletal structures and, to some degree, outlines of organs. X-ray methods, however, do not clearly detect the boundaries of softer tissues such as muscles, brain, and tumors. It must be used sparingly, inas much as excessive exposure to radiation adversely affects living organisms.

Engineers and physicians working together have been searching for a safe, external method to display body structures pictorially. Remarkable success has been achieved by exploiting acoustic energy (sound) as used in sonar systems to detect and track solid objects under the sea. One form of acoustic scanning, called echo-sonography, transmits high frequency waves into a sound-conducting medium and measures the time required for signals to reach and echo back from the reflecting surface. Given the direction of the transmitted beams and the acoustic velocity of the medium traversed, one can record depth and dimensions.

Ultra-high frequency sound waves directed into the body echo with different intensities from various tissues. Sound waves at frequencies much higher than those audible to the human ear are required; typical frequencies for medical use range from 1,000,000 to 10,000,000 cycles per second. The upper limit for human hearing is approximately 20,000 cycles per second.

Ultrasonic waves produce images similar to those of X rays. Signals pass through the body, with hard structures conducting sound energy to a greater extent than soft tissues. The sound waves provide diagnostic information otherwise unobtainable. They can distinguish many different kinds of tissue, examine a fetus inside a mother's body with no harm to either, and detect tumors, lesions in the eye, gallstones, benign cysts and kidney and liver abnormalities.

A team of specialists from the National Heart and Lung Institute, the Division of Research Services and the National Aeronautics and Space Administration recently developed and improved an ultrasonic system for non-invasive visualization of heart structure to evaluate effects of prolonged weightlessness on astronauts, by recording dimensions of astronauts' heart muscles just prior to lift-off, and immediately after splashdown, during the Skylab III flight. (See NHLI section on Echocardiography in Cardiovascular Diagnosis.)

Closing in on Laboratory Animal Disease

Stimulated by the needs of other science programs, the Division makes significant research contributions of its own.

In 1917, Ernest Tyzzer, a scientist working with mouse tumor transplants, encountered and reported a fatal disease of the liver which destroyed his laboratory colony. The disease became known as Tyzzer's disease and was a principal cause of death in mouse colonies, interfering drastically with biomedical research. In 1965, Dr. Anton M. Allen, a DRS veterinarian, diagnosed Tyzzer's disease in an

epidemic in which 50 percent of a group of weanling rabbits died. His published report catalyzed interest and led to the recognition of Tyzzer's disease in rats, hamsters, gerbils, cats, horses and nonhuman primates. Recently it also has been identified as identical to Arrington's disease, a fatal scourge of wild muskrats.

The causative agent, *Bacillus piliformis,* remains unclassified. The parasite is unlike other known organisms in microbiology because of its unique combination of characteristics. All attempts to culture it in artificial media failed until another DRS veterinary microbiologist, Dr. James R. Ganaway, using unorthodox methods, successfully isolated and propagated the organism in chicken eggs in the late 1960's.

Antibiotics used therapeutically or as a prophylaxis are of questionable value against Tyzzer's disease, since environments usually are contaminated with spores of the organism. Special techniques such as silver impregnation of tissue and fluorescent antibody stains are helpful in detection. Though much work is yet to be done, enough is known that development of a vaccine realistically can be anticipated.

Tyzzer's disease is a leading cause of mortality in animal colonies throughout the world. There has been speculation as to association of *Bacillus piliformis* with diarrhea, colitis, and other intestinal disease of unknown causes in other species, including man. This speculation seems reasonable, inasmuch as Tyzzer's disease is distributed world-wide in many animal species, the causative agent probably survives in nature as spores, and is easily transmitted in spore-contaminated food.

The extent to which Tyzzer's disease interferes with biomedical research is unknown. Often, it is belatedly diagnosed after diarrhea fails to respond to antibiotics. It is often misdiagnosed at autopsy.

The recent findings of DRS scientists, and the availability of the DRS specialized germ-free environment and isolation barrier techniques, are encouraging indications that the means of control and eliminate the disease will be found.

International Collaboration on Brain Research

Understanding the brain's reaction to oxygen deprivation has important implications for several medical problems, including carbon monoxide poisoning, hepatoencephalopathies (liver diseases accompanied by brain abnormalities), head injury and stroke. In studying the brain's responses, Dr. Igor Klatzo and other scientists of the National Institute of Neurological Diseases and Stroke, collaborating with investigators at the Polish Academy of Sciences under the Fogarty International Center's Special Foreign Currency Programs, have focused on two main brain-tissue components: nerve cells (neurons), which by means of impulses send information to other neurous or to organs of the body; and glial cells, which supply nourishment and energy to neurons. Glial cells also help form and maintain sheaths that insulate nerve fibers, enabling each of them to conduct electrical impulses individually. In addition, one type of glial cell removes brain tissue that has disintegrated as a result of disease or trauma.

These scientists have helped explain normal function of nerve and glial cells and that of the entire brain. They showed how these functions may be altered by disorders that impede or completely cut off the brain's blood supply and thereby deprive the cells of oxygen and nutrients.

The scientists were able to explain, for example, how liver disease can cause brain abnormalities. Normally, the liver converts ammonia, one of the byproducts of protein metabolism, to a less toxic form before it reaches the brain in the blood stream. A diseased liver, however, may not convert ammonia. When the ammonia enters the brain, the scientists found, it destroys both glial and nerve cells.

The scientists demonstrated in tissue culture studies that carbon monoxide alone can injure nerve and glial cells. Previously, it was believed that the brain injury in cases of carbon monoxide poisoning was caused only by oxygen deprivation; carbon monoxide diminishes the amount of oxygen that the blood's hemoglobin can carry from the lungs.

The collaborators also found a change in glial cells that is the most sensitive indicator of metabolic injury to brain tissue. This change is an accumulation of glycogen, the stored form of glucose, the basic brain nutrient. When newborn monkeys were subjected to asphyxia (suffocation), glycogen levels rose sharply in the brain, and accumulated in glial cells.

The studies of nerve cell survival and recovery following deprivation of blood supply have important implications for helping stroke patients in the critical period directly following the interference with the cerebral blood supply producing the stroke. Small rodents subjected to brief blood-supply deprivation and sacrificed less than one week later showed extensive nerve cell changes. Those surviving longer than one week showed only small, confined areas of brain injury, indicating that cell recovery may take place during that critical period.

Another type of change leading to total recovery was observed in the Ammon's horn, that part of the brain most sensitive to ischemia (deprivation of blood). A Polish investigator, Professor Miroslaw Mossakowski, working at NIH, found that neurons affected by ischemia are very active metabolically, showing a striking increase in the number of enzymes directly involved in utilizing oxygen. This finding provides new insight into the mechanisms of recovery for these oxygen-deprived cells.

The scientists are also studying shark brains to learn how nerve and glial cells recover following cessation of blood supply. Sharks, which are low on the evolutionary scale, have an extremely low metabolic rate, and the blood vessels in their brains have unusual permeability properties. Preliminary experiments revealed that the shark brain can fully recover from total interruption of blood supply for as long as 90 minutes. The scientists are now trying to determine how the cells recover, and whether these important changes could be applied to studies of the human brain when deprived of its blood supply by injury or stroke.

Scholars-in-Residence

A relatively new activity within the National Institutes of Health is the Fogarty Scholars-in-Residence Program. Scholars from the United States and other countries are invited to spend from three to twelve months in residence in Bethesda to engage in writing, research and lecturing, in addition to inter-

acting with the NIH scientific staff. During the past four years, more than 35 distinguished scholars have participated in this program.

One of these recent visitors was the eminent chemical immunologist, Professor Michael Sela of the Weizmann Institute of Science, Rehovot, Israel. Dr. Sela's research has focused on the intriguing subject of synthetic antigens, substances capable of stimulating specific defense responses in an organism. Until 12 years ago, the only available antigens were of natural origin. Dr. Sela's laboratory was the first to prepare synthetic antigens, which when injected into experimental mice, produced the antibodies that are a part of the immune response in bodily defense. Since that significant achievement, Dr. Sela has succeeded in producing a large number of synthetic antigens. His success suggests that vaccines of the future, whether viral or bacterial, may be prepared by chemists rather than by biologists.

While a Fogarty Scholar-in-Residence, Dr. Sela wrote several scientific articles on chemical immunology. He was in almost daily contact with scientific colleagues at NIH, initiated and participated in many seminars, and presented a number of formal lectures, including the prestigious NIH Lecture in Bethesda.

Dr. Sela's extensive involvement with young scientists as well as established investigators provided a stimulus of ideas in the broad field of immunology, an area of intense interest to many NIH researchers.

Another participant was Dr. Elvin A. Kabat, Professor of Microbiology and Human Genetics and Development, Columbia University School of Medicine, who is well known for his contributions to immunology and immunochemistry. As a Fogarty Scholar, Dr. Kabat revised and updated his publication, "Structural Concepts in Immunology and Immunochemistry," which is used in medical schools, graduate schools, and undergraduate courses in microbiology, immunology and immunochemistry.

Dr. Kabat is concerned with future directions for immunological and immunochemical research, especially studies on the structure of immunoglobulins and antibodies, the blood components involved in the body's defensive responses to challenging antigens.

Dr. Kabat conducted computer studies in cooperation with specialists in the Division of Computer Research and Technology, and conferred continually with cellularly-oriented scientists, particularly those studying antibody-combining sites. His expertise and interests involved him in problems associated with virus hepatitis antigens, an area of grave concern. During his residence at NIH, he was closely associated in exchange of ideas with other Scholars, including Professors Osamu Hayaishi, Lars Ernster and Michael Sela and Drs. Paul Zamecnik and Dirk van Bekkum.

A critical aspect of the research at the National Institutes of Health is the part played by young physicians. For some 20 years since the opening of the Clinical Center and the expansion of the National Institutes of Health intramural program into clinical problems, physicians have been coming to NIH as part of their training. In general, they come after an internship and a year of residency, and spend two or three years. This NIH experience has several unique and important aspects.

In the first place, it provides one of the few situations in which a young physician can simultaneously participate in medical care, learn new clinical skills, and carry out extensive and detailed investigation in the laboratory. The Clinical Center was specifically designed so that the laboratories in which clinical research is carried out are adjacent to patients' rooms. Every convenience is offered for simultaneously taking care of patients and carrying out biochemical investigations on their problems.

The second particularly important aspect of training in research medicine as it occurs at the NIH is the nature of the patient population. All patients are referred by their private physicians and there is an obligation to admit only those who fit into a research protocol. In addition, since there is no charge for patient care, there is no obligation to make diagnosis and therapy as rapid as possible so that the patient can be discharged promptly for economic reasons. This means that it is possible to study patients without the pressure of time and economic considerations.

Additionally, approximately 10 to 15 percent of the "patients" are normal volunteers, who serve as controls to validate the research. These volunteers frequently are students who themselves spend some time in the laboratory and who appreciate to a considerable degree the exact significance of procedures being performed.

Finally, the hospital at the National Institutes of Health is unique in having enormous representations of certain diseases and perhaps none at all of other diseases. This means that someone interested in a particular aspect of, say, arthritis, will see an unusually large number of patients with the rarest forms of the disease. Obviously, as training in medicine, this is highly specialized and requires supplementation with broader clinical experience. A recent development has been increasing interaction among the Institutes, perhaps exemplified best by a clinical training program in endocrinology in which the Clinical Associates rotate among the wards of four Institutes.

Another important consideration for a young physician interested in academic medicine or clinical investigation is the location of basic science laboratories in the hospital building itself. It is possible for a physician doing clinical investigation to talk with biochemists, physical chemists, spectroscopists, or others

who may only be a few feet—or at most a few floors—from his own laboratory.

Let us look at a typical history of a young man who comes to the National Institutes of Health. Usually Dr. Smith will have had his first experience in laboratory work as an undergraduate when he spent several summers working with a professor. In medical school, Dr. Smith took a summer and an elective period working in another laboratory, so that by the time he was graduated he had some knowledge of laboratory procedures and some interest in research. Nonetheless, Dr. Smith had a busy internship and a year of residency at which time his career plans were not complete, but included a rather vague wish to consider academic medicine where he could participate in patient care but also would have an opportunity for teaching and for some research.

In order to get a better feeling for how important research would be to his career, he decided to spend two years at the National Institutes of Health and was accepted as a Clinical Associate. Here he spent nine months full time on the wards, taking care of patients and providing consultation to other services. In addition, he worked with a Ph.D. in biochemical aspects of the diseases he and the group with which he worked were particularly

interested in. After about a year and a half it became apparent that although he had several important leads, he really wouldn't be able to finish the research as he would like to in two years, and he obtained a third-year appointment. Before this was completed, it became clear to him and some of his associates that yet another year would substantially advance what they were trying to do, and so he stayed a fourth year. By that time, he had offers as an Assistant Professor at two different universities and accepted the most attractive of these as a starting point in his academic service.

Approximately 75 percent of the Clinical Associates at the NIH follow this career outline. Perhaps 10 percent stay on for five or more years. A few Associates elect private practice; some go into basic science and join the staff of medical schools as full professors of biochemistry, microbiology, or other scientific subjects.

Whatever his eventual career choice, the young physician finds special challenges and opportunities during his Bethesda years. Concentration on excellence and level of productivity has given NIH intramural research a special image in public and private institutions, learned societies and health interest groups.

It has been a source of satisfaction to NIH leaders to find that many external elements do not regard research at the National Institutes of Health with envy or as a competitor for funds. Rather, each new constituency seeking an enhanced focus of visibility, or creation of a new categorical Institute, has emphasized the importance of an intramural NIH component in developing research concepts of benefit to a total national field, in providing training of unmatched quality, and in the yardstick that rigorous intramural standards provide for the evaluation of extramural research.

Accordingly, an appointment at NIH allows the young investigator to become a part of an organization devoted exclusively to research, with no teaching responsibilities. About half of the more than 10,000 full-time employees on the attractive Bethesda site are engaged in direct research—a concentration not found elsewhere. The budget for intramural research is approximately $110 million.

A particular source of pride for a young researcher can be the opportunity for association with individual scientists who command unusual respect in the scientific community. There are many forms of recognition that such respect takes, including the recording of progress in some 3,500 published scientific articles each year by the intramural staff, and the recognition afforded by honors, awards, prizes and prestigious appointments. His day-to-day colleagues may include recipients of the National Medal of Science, the Rockefeller Public Service Award, the Lasker Award, the Nobel Prize or other honors attesting to the unique

structure of the largest biomedical research organization in the world.

The junior workers who arrive at NIH in a steady flow of 300 or 400 each year also join senior Visiting Scientists and Fellows, attracted by the opportunity for intensive research experience and the reputations of the NIH staff.

Most importantly, they find an opportunity to contribute specifically to the scientific knowledge base, and after a few years typically move on to cross-fertilize the scientific world with methods and ideas generated in NIH research, as holders of prestigious lifetime positions in academic medicine.

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